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Transgenerational Transfer of Dietary Lipids and its Consequences for Offspring Physiology in a Marine Teleost

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Dedication

To my family.

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Abstract

Transgenerational Transfer of Dietary Lipids and its Consequences for Offspring Physiology in a Marine Teleost

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Maternal provisioning of nutrients can play a key role in the growth, development and long-term fitness of offspring. In teleosts, the composition of maternally derived nutrients (in yolk and oil) depends partly on maternal diet. This dissertation investigates the process of maternal-offspring nutrient transfer and the transgenerational effects of maternal nutrition on offspring physiology in a marine teleost, red drum (*Sciaenops ocellatus*). Results of 21 diet-shift experiments, from which the fatty acid profiles of the diets and eggs were compared, showed that 15 fatty acids in eggs were correlated with their levels in the recent diet, and the rate of incorporation of fatty acids into eggs was proportional to the magnitude of the diet shift. Further assessment revealed that maternal diet affected egg triglyceride (TG) content but not other lipid classes. Parental dietary variations rapidly affected the fatty acid composition of all major lipid classes in eggs (TG, wax ester/steryl ester (WE/SE), and phosphatidylcholine (PC)), with greater effects on neutral lipids (TG, WE/SE) than on the polar lipid (PC). Rates at which embryos and larvae utilized 15 fatty acids varied with maternal diet and were proportional to their initial concentrations in eggs. Rates of utilization of the oil globule were also affected by maternal

diet, resulting in differences in oil globule size at the first feeding stage. Effects of different maternal diets on larval fatty acid compositions persisted at the time of onset of exogenous feeding and afterward. In addition, prolonged effects of maternal nutrition on larval lipid metabolism (nutritional programming) were documented. At 21 days post-hatching (dph), larvae reared under common conditions from eggs produced by adults on different diets showed differences in total fatty acid accumulation and fatty acid profiles (especially polyunsaturated fatty acids in TG). Collectively, these research findings demonstrate some of the profound transgenerational effects of maternal nutrition on offspring physiology. The altered lipid metabolism of offspring resulting from variations in parental diet may have consequences for larval physiological processes and behavioral performance, which may ultimately influence their survival and fitness.

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Chapter 1: Introduction

Embryonic and early larval development and metabolism are fueled entirely by maternally derived nutritional resources (yolk and oil) before the onset of exogenous feeding. In teleosts, the composition of these maternally derived nutrients depends partly on maternal diet (Wiegand 1996; Johnson 2009; Jaroszewska and Dabrowski 2011), but the degree to which egg nutrient profiles reflect maternal diet varies among species. Maternal provisioning of nutrients can influence the growth and development of offspring (Rainuzzo et al. 1997; Izquierdo et al. 2001). These effects sometimes extend beyond the early ages and lead to long-term consequences. This transgenerational connection highlights the importance of maternal nutrition to offspring physiology and performance, but it is not well understood.

EARLY LIFE STAGE AND MATERNAL NUTRITION

The early life stages of fishes are a critical developmental window during which structures and functions of organ systems begin to develop (Fuiman 2002). During this period of rapid cell proliferation and differentiation, offspring exhibit dramatic morphological and physiological changes and great developmental plasticity in response to extrinsic factors, such as temperature, light, and nutrients (Pittman et al. 2013). The larval stage is also a period of high mortality, with an estimated 99.9% mortality rate due to starvation, predation, and diseases (Houde 2002). Among the numerous environmental and biotic factors that contribute to the recruitment variations are the non-genetic maternal effects that contribute to the phenotypic variations in offspring (Green 2008). One form of maternal investment is the nutritional provisioning through eggs.

Female fishes draw upon dietary intake, body stores, or *de novo* synthesis to supply nutrients to eggs (Wiegand 1996; Johnson 2009). The extent to which female fishes rely on each of these sources depends on their reproductive and resource allocation strategies. Some fishes use previously accumulated nutrients in the body stores to supply the eggs with nutrients needed by the offspring. These fishes are represented by migratory, semelparous species that decrease or stop feeding prior to spawning, and can be considered capital breeders (Jönsson 1997; McBride et al. 2015). Batch spawning fishes that continue to feed through sexual maturation and spawning largely derive the nutrients from recently ingested diets (Johnson 2009; Fernández-Palacios et al. 2011), and therefore, can be considered income breeders. Many fishes adopt an intermediate breeding strategy within a continuum between the two extremes (McBride et al. 2015).

LIPID BIOCHEMISTRY AND LIPID METABOLISM IN FISHES

Lipids and constituent fatty acids are an important class of nutrients for fishes, and are present in high concentrations in fish eggs. This diverse group of molecules can be divided into neutral and polar lipids (Sargent et al. 2002; Tocher 2003). Polar lipids consist of phosphoglycerides and sphingolipids[1]. Phosphoglycerides contain two fatty acids esterified to a backbone of phosphatidic acid (PA), and neutral lipids are mainly composed of triglycerides (TG), wax esters (WE), and steryl esters (SE).

Lipids have a number of important roles in fish physiology. Neutral lipids and constituent fatty acids are the primary energy source as they have twice the caloric density of carbohydrates and proteins (Tocher and Glencross 2015). During events with high energy demand, such as starvation and reproduction, lipids (especially TG) are mobilized

[1] Phospholipid is a more general term comprising lipids with phosphorus, including phosphoglycerides and sphingomyelin (SM)).

from the lipid depots (Tocher 2003). Polar lipids are important structural components and are incorporated into the cellular membranes. The fatty acid compositions of membrane phospholipids are critical for maintaining the physical properties (e.g., fluidity) and the functions of cellular membranes and membrane-bound enzymes (Tocher 2003). Some tissue-specific patterns, such as the accumulation of docosahexaenoic acid (DHA; 22:6n-3) in the central nervous system (Lauritzen et al. 2016), indicate that fatty acids serve critical roles in proper development and system function, and that tissue fatty acid compositions can have key influences on an organism's fundamental physiological processes.

Metabolism of lipids provides phosphate for the synthesis of nucleic acids, choline for neurotransmitters, and sterols for steroid hormones (Rainuzzo et al. 1997; Tocher 2003). Furthermore, lipids and their derivatives (e.g., PA, phosphoinositides – derivatives of phosphatidylinositol) are also responsible for a range of intra- and inter-cellular signaling pathways. Polyunsaturated fatty acids (PUFA) and cholesterol are involved in the regulation of gene transcription, mainly for genes involved in the lipid homeostatic processes, by acting as ligands for nuclear receptors and transcription factors (Tocher 2003). Some PUFA, arachidonic acid (ARA; 20:4n-6) and eicosapentaenoic acid (EPA; 20:5n-3), are also precursors of eicosanoids, which are biologically active molecules important for modulation of immune response, inflammatory response, renal function, and neural function (Tocher 2003). The regulation of lipid homeostasis involves a sequence of molecular events and metabolic pathways, including uptake, transport, storage, biosynthesis, metabolism, and catabolism (Ferramosca and Zara 2014; Tocher and Glencross 2015).

Marine fishes contain high levels of n-3 PUFA, especially fish eggs, in which n-3 PUFA frequently approach 50% of the total fatty acids (Tocher and Sargent 1984).

However, all vertebrates lack Δ12 and Δ15 desaturases to form 18:2n-6 and 18:3n-3 from 18:1n-9 (Tocher 2003). Therefore, these two C18 PUFA need to be obtained through the diet. Furthermore, marine fishes have limited capabilities to synthesize long-chain PUFA from these C18 precursors, which is attributed to deficiencies in one or more key enzymes of the long-chain PUFA biosynthetic pathway (Monroig et al. 2018). Recent evidence, however, has shown that some marine fishes may not completely lack this biosynthetic capability, although great interspecific variation exists (Monroig et al. 2018).

EFFECTS OF VARIABLE EGG COMPOSITIONS

There is considerable interspecific variation in egg lipid compositions and larval utilization of endogenous lipids in fishes (Kaitaranta and Ackman 1981; Sargent et al. 2002). Yolk consists mainly of lipoproteins, while discrete oil globule(s) contain mostly or entirely neutral lipids (Wiegand 1996). Therefore, eggs with oil globule(s) are generally rich in lipids (>15% of dry weight), predominantly neutral lipids, which are utilized as the main metabolic fuel during embryonic and early larval development. On the other hand, eggs without oil globule(s) contain relatively lower lipid content (<15% of dry weight), and embryos and larvae use polar lipids and amino acids as major energy substrates (Tocher 2003; Jaroszewska and Dabrowski 2011). Catabolism of yolk and oil lipids releases free fatty acids (FFA) that are used for energy production or reacylated to form developing tissues (Tocher 2003). In general, MUFA are preferentially catabolized while PUFA are retained (Wiegand 1996; Tocher 2003).

It had previously been thought that the egg compositions are generally stable within a species to ensure proper development of offspring (Dalsgaard et al. 2003a). While egg compositions may be relatively resistant to variations in external factors compared to those of body tissues (Tocher 2003), mounting evidence has shown egg fatty acid composition

varies in response to changes in maternal diets, particularly for species that are identified as income breeders (Mourente and Odriozola 1990; Harel et al. 1994; Furuita et al. 2002; Li et al. 2005; Fuiman and Faulk 2013). Given the high energetic demand during reproduction, there could be competing demands from maternal somatic tissues and developing oocytes (Wiegand 1996). Female metabolism of ingested and stored lipids before deposition into oocytes further complicates the transfer process (Galloway and Budge 2020). For example, studies have revealed selection for some HUFA (e.g., DHA) and against MUFA (e.g., 22:1n-11) during lipid mobilization from muscle tissue to oocytes (Henderson et al. 1984; Garrido et al. 2007). Therefore, considering the distinct reproductive strategies, ovarian development patterns, and egg compositions among species, the maternal-offspring lipid transfer processes should be considered species-specific.

Proper maternal nutrition is crucial to embryonic and larval survival and development during the endogenous feeding period. Studies of various species have shown that dietary levels of n-3 high-unsaturated fatty acids (HUFA) are positively correlated with various egg quality parameters, such as fertilization success, hatch rate, and viability (Harel et al. 1994; Rainuzzo et al. 1997; Sargent et al. 2002). As a result, nutritional compositions (lipid content, fatty acid compositions) of maternal diets have received much research attention, particularly motivated by aquaculture studies aimed at improving egg and larval production (Rainuzzo et al. 1997; Izquierdo et al. 2001). However, the mechanism through which maternal nutrition contributes to the success of offspring via yolk and oil composition remains poorly studied. Therefore, further research is needed to understand the impact of differences in egg compositions, in response to variations in maternal diets, on larval development and utilization of yolk and oils.

The effects of maternal nutrition can sometimes extend beyond yolk and oil exhaustion. The association between the early nutritional environment and long-term consequences is termed nutritional programming. Nutrition programming has been primarily studied in mammals due to its association with human health, such as metabolic diseases (Symonds et al. 2009; Ozanne 2015). In recent years, it has been studied in other animals including fishes, and consequences for growth, neural development and nutrient metabolism have been documented in fishes (Vagner et al. 2007; Lund et al. 2012; Morais et al. 2014; Fuiman and Perez 2015; Izquierdo et al. 2015). Several studies have found nutritionally suboptimal diets (plant-based diets that are deficient in HUFA, or high-carbohydrate diets) provided to the larvae at first feeding improved acceptance or utilization of those diets at later stages (Geurden et al. 2007, 2014; Gong et al. 2015; Rocha et al. 2016a, b; Perera and Yúfera 2016a, b; Zambonino-Infante et al. 2019).

Overall, understanding of nutritional programming in fishes remains limited. Existing studies have largely focused on the effect of early (larval) diet, and are limited to several species. Only a handful of studies have looked at programming via maternal nutrition. These studies have shown that differences in maternal nutrition, which translate to altered egg compositions, could have consequences for offspring growth, metabolism, and behavioral performances several weeks after yolk absorption (Fuiman and Ojanguren 2011; Morais et al. 2014; Fuiman and Perez 2015; Burns and Fuiman 2019). For example, a study of red drum (*Sciaenops ocellatus*) showed that DHA content of larvae was correlated with levels of DHA in the eggs, even when larvae were raised on a DHA-replete diet for several weeks (Fuiman and Perez 2015). Furthermore, the differential accumulation of DHA by larvae was associated with aspects of larval performance that are critical to survival, such as growth, routine swimming, and predator escape response (Fuiman and Perez 2015). Dietary lipids and fatty acids are important for larval survival, growth, stress

resistance, swimming, feeding, and escaping behavior (Hamre et al. 2013), and nutritional programming highlights a unique mechanism through which maternal nutrition could alter how a larva metabolizes or utilizes its ingested nutrients. This could occur through physically altering or impairing the ontogenetic development of somatic structures (e.g., digestive tract, hypothalamus), or alternatively, through limiting or irreversibly switching certain metabolic pathways on or off (Lucas 1991; Pittman et al. 2013). Studying the effects of maternal nutrition on important physiological processes of offspring may provide insight into the consequences and mechanisms of nutritional programming, but such studies remained scarce.

The research presented in this dissertation aims to deepen our understanding of the role played by maternal nutrition in mediating the well-being of offspring. This parent–progeny relationship was investigated in two parts: the process of maternal-offspring nutrient transfer, and the transgenerational effects of maternal nutrition on offspring physiology. The objectives of this research follow a logical maternal-offspring progression: (1) to examine the dynamics of transfer of lipids and fatty acids from maternal diet to eggs; (2) to examine the utilization of endogenous lipids and fatty acids by embryos and early larvae under the influence of different maternal nutrition; and (3) to determine the effects of variations in the maternal nutrition on lipid metabolism of larvae at later stages.

The first and second studies investigated the maternal transfer of dietary lipids and fatty acids to eggs in red drum. By conducting a series of diet-shift experiments, the first study aimed to identify fatty acids in the eggs that respond predictably to variations in maternal diet, and assess the temporal dynamics of diet-egg transfer by estimating the lag between ingestion and incorporation into eggs, and the rate of incorporation of fatty acids into eggs. The second study further assessed the maternal dietary effects on egg

compositions by examining the transfer of lipid classes and addressing whether the dietary effects on egg fatty acid composition pertain to particular lipid classes. In the third study, different diets were fed to red drum broodstock to alter egg compositions, and the utilization patterns of lipids and fatty acids were recorded to determine the effects of maternal provisioning of nutrients on larval lipid metabolism during the endogenous feeding period. In the fourth study, larvae from spawns that derived from several distinct maternal diets were raised until 21 days post-hatching under common conditions. The effects of differences in the maternally derived egg compositions on lipid composition on larvae were assessed to investigate the transgenerational effects of maternal nutrition on offspring physiology, with the goals of identifying the nutritional stimulus responsible for nutritional programming and the consequences for larval lipid metabolism.

Chapter 2: Dynamics of diet-egg transfer of fatty acids in the teleost fish, red drum (*Sciaenops ocellatus*)[2]

INTRODUCTION

Egg boons – dense patches of eggs produced by coordinated spawning events of marine populations – are a rich but ephemeral nutritional source for egg consumers. Essential fatty acids (EFA) are tens to hundreds of times more concentrated in eggs than other particles of their size in the sea (Fuiman et al. 2015a). Therefore, egg boons, especially those produced by marine fishes, may be important for understanding temporal and spatial variations in food webs because they represent an important pathway for the counter-gradient flow of energy and EFA from higher to lower trophic levels (Fuiman et al. 2015a).

Fatty acids (FA) present in fish eggs derive from three possible sources: recent ingestion, somatic reserves (with some level of modification), and *de novo* synthesis within the ovary (Wiegand 1996; Johnson 2009). The proportion of egg FA that comes from each source varies depending on the species' reproductive strategy. Batch-spawning fishes that continue to feed during spawning periods largely depend on recent diets for nutrients that are deposited in eggs (Johnson 2009; Fernández-Palacios et al. 2011), which provides an opportunity to understand trophic flow of FA through egg boons. A recent field study, for example, reported substantial interannual variation in FA composition of eggs of a batch-spawning marine fish and demonstrated that this variation was associated with climate-driven changes in prey availability (Fuiman 2018).

[2] This chapter has been published: Hou Z, Faulk CK, Fuiman LA. 2020. *Phil. Trans. R. Soc.* B 375: 20190646. http://dx.doi.org/10.1098/rstb.2019.0646. ZH participated in the design of the experiments, collection and analysis the samples, interpretation of the data, and prepared the manuscript.

Our current study examines the transfer of FA from maternal diet to eggs in a marine batch-spawning fish. The purpose is to identify which FA in eggs respond predictably to variations in maternal diet and the temporal dynamics of diet-egg transfer. This information will improve our ability to infer maternal diet from FA composition of egg boons and thereby clarify trophic linkages that lead to egg consumers.

MATERIAL AND METHODS

We conducted two sets of diet-shift experiments on a batch-spawning marine fish, red drum (*Sciaenops ocellatus*). In one set of experiments, five broodstock tanks (identified as MT1, MT6, MT7, MT8, and MT9) were given multiple diet shifts to estimate the lag between ingestion and incorporation into eggs – time (days) it takes for a dietary change in FA content to appear as a change in FA content in the eggs. In the other set of experiments, six broodstock tanks were given a single diet shift to estimate the rate of incorporation of ingested FA into eggs. Each individual diet shift in the first set of experiments was also treated as a single diet-shift and combined with data from the second set of experiments, resulting in 21 individual diet-shifts (details see Appendix A). Eggs were collected before and after diet shifts and concentrations of 27 FA (representing 91.1 to 98.2% of total FA) were examined and related to dietary FA. Selected sums and ratios of FA that have been used as trophic markers were also calculated. Unless otherwise noted, methods and analyses followed those of our previous study of arachidonic acid (ARA, 20:4n-6; Fuiman and Faulk 2013).

Sample collection

Nine types of feed were used to create 17 different diets (details see Appendix A and Tables A1 & A2). Adult red drum were fed 3 to 5 days per week (for a daily feeding

record, see a separate file Supplementary Document D1) and were induced to spawn naturally by manipulating temperature and photoperiod (Arnold et al. 1977). Spawning occurred in the evenings every 2 to 8 days and eggs were sampled approximately 12 to 16 h post-fertilization from almost every spawn (samples are listed in a separate file Supplementary Document D2), rinsed with deionized water, and stored at -80°C until analysis. Diet items were also sampled regularly for FA analysis. FA composition of eggs and diet items (expressed as mg g^{-1} dry weight) were measured by gas chromatography using established methods (Faulk and Holt 2005; details see Appendix A).

Lag analysis

Data from the multiple-diet-shift experiments were used to estimate the temporal lag between ingestion and incorporation into eggs. Intake of each FA ($Intake_{FA}$; mg $fish^{-1}$) at each meal was calculated as:

$$Intake_{FA} = \frac{\sum_i WW_i \times DW_i \times [FA_i]}{n},$$

where WW_i is the wet weight (g) of diet component i fed to a broodstock tank (recorded at each meal), DW_i is the ratio of dry weight to wet weight of diet component i, $[FA_i]$ is the concentration of FA in diet component i (mg g^{-1} dry weight), and n is the number of fish in the tank. A time series of mean daily dietary intake of each FA (mg d^{-1} $fish^{-1}$) was constructed using the moving average of $Intake_{FA}$ of the seven preceding days, since the broodstock feeding schedule was repeated on a weekly basis.

Pearson's correlation coefficients (r) were calculated relating the amount of each FA in all eggs samples (mg g^{-1} dry weight) to the time series for mean daily dietary intake for the same FA (mg d^{-1} $fish^{-1}$) for lags of 0-30 days. That is, correlations between these two data sets were computed for lags of 0 (eggs correlated with dietary intake on the same day) through 30 (eggs correlated with dietary intake 30 days earlier). This generated 31 r

values for each FA in each experiment. The 31 *r* values were plotted against lag for each FA in each data set (broodstock tank) to ensure there was a progressive trend toward the maximum (i.e., maximum *r* was not random) and the lag corresponding to the maximum *r* was determined for each FA in each experiment (Figure A1; details see Appendix A). A FA was determined to be a useful trophic marker if the maximum *r* was positive and statistically significant (one-tailed test, $P < 0.05$) for at least 4 of the 5 multiple-diet-shift experiments. The median of the four or five lags was taken as the estimate of the lag (time between ingestion and incorporation into eggs).

Incorporation rate analysis

Turnover of FA in fish tissues during growth has been shown to follow a simple dilution model (Robin et al. 2003; Jobling 2004), which predicts that the rate of change of a FA is directly proportional to the magnitude of its change in the diet. The same model appears to adequately describe the changes in the ARA level of red drum eggs following a diet shift (Fuiman and Faulk 2013). Here, we tested whether the incorporation of each FA following a diet shift is directly proportional to the magnitude of the diet change for the same FA.

For each of the 21 single-diet-shift experiments, the magnitude of change in dietary intake (ΔFA) for each FA was calculated as the difference between the means of dietary intake 28 d after and 28 d before the diet shift. FA level in eggs generally exhibited a linear trend after a diet shift. Therefore, the rate of incorporation of each FA ($I_{FA,}$ expressed as mg g^{-1} dry weight d^{-1}) into eggs was estimated for each diet shift as the slope of a linear regression between egg FA concentration and time since the diet shift (days) for all spawns that were sampled between 0 and 32 days after the diet shift (Figure 2.1; more details see Appendix A). A significant linear relationship between the rate of incorporation and the

magnitude of diet shift (I_{FA} on ΔFA) means that the simple dilution model can be used as a quantitative model to describe the change in concentration of a FA over time following a diet shift.

RESULTS

Lag analysis

Fourteen of the 27 measured FA in eggs were positively correlated ($p < 0.05$) with their respective levels in the adult diet in at least four of the five multiple-diet-shift experiments (Figures 2.2 & 2.3). In addition, there was a significant correlation between eggs and diet for 22:1n-11 when the diet shift was sufficient (Δ22:1n-11 ~1500 mg d^{-1} $fish^{-1}$ in MT1 vs. 18-55 mg d^{-1} $fish^{-1}$ in MT6, MT8 and MT9; Figure 2.2c). Nine sums or ratios showed positive correlations between eggs and diet (Figure 2.3). Lags for these 15 FA [1 saturated FA (SFA), 2 monounsaturated FA (MUFA) and 12 polyunsaturated FA (PUFA)] and 9 sums or ratios ranged from 2.5 to 17 days (Figure 2.3). Levels of 12 FA and 4 sums and ratios were positively correlated ($p < 0.05$) with their respective levels in the adult diet for 1 to 3 out of the 5 multiple-diet-shift experiments (Figure 2.3) and, therefore, those FA in eggs were not considered to be strongly related to diet.

Incorporation rate analysis

Incorporation rate of FA into eggs followed predictions of a simple dilution model for 11 FA, as evidenced by significant regressions of I_{FA} on ΔFA (Table 2.1; Figure 2.4). These 11 FA included two SFA, one MUFA, and eight PUFA. Incorporation rates for (n-3) and (n-6) PUFA and highly unsaturated FA (HUFA)[3] and their respective ratios were also strongly correlated with the magnitude of diet shift.

[3] PUFA refers to FA with ≥ 2 ethylenic bonds. HUFA refers to FA with ≥ 20 carbon atoms and ≥ 3 ethylenic bonds.

DISCUSSION

The fatty acid trophic marker (FATM) method has been applied to achieve a qualitative understanding of spatial and temporal variations in primary production and consumer diets in marine ecosystems (Dalsgaard et al. 2003b). Quantitative assessment of the diet composition of higher trophic levels has been constrained by a lack of understanding of consumer modification of dietary FA (Dalsgaard et al. 2003b). Quantitative fatty acid signature analysis (QFASA; Iverson et al. 2004) provides a quantitative assessment of trophic interactions and has been applied to high level predators, such as seabirds and marine mammals (Budge et al. 2006; Iverson 2009), but application of FA biomarkers to quantitatively study diets of fishes remains relatively rare (Budge et al. 2012; Happel et al. 2016). Furthermore, no studies have used eggs as source material. It had been thought that the adult fish diet would have little impact on FA composition of eggs because embryos and larvae require specific nutrition for proper development (Dalsgaard et al. 2003b). However, laboratory studies have shown that FA composition of fish eggs varies in response to changes in adult diet (Harel et al. 1994; Almansa et al. 1999; Furuita et al. 2002; Li et al. 2005). In this study, we identified 15 FA and 9 sums or ratios in eggs that respond predictably to variations in maternal diet. This information could be used to inform the application of FATM and QFASA as to which FA in eggs may be useful trophic markers.

Maternal metabolic modification

Understanding the physiological pathways through which ingested FA are modified leads to a better understanding of the results of this study and their applicability to other species. Between ingestion and incorporation into eggs, consumers can distort dietary FA signals through biosynthesis (*de novo* or desaturation/elongation) and selective uptake,

mobilization or oxidation (Dalsgaard et al. 2003b). Although it has been argued that oxidation of FA (including PUFA) increases with higher greater dietary availability (Tocher 2015), oxidation of PUFA for energy may be less likely during reproduction when there is high demand for incorporating these FA into eggs. Indeed, it has been shown that some PUFA are preferentially retained and incorporated into oocytes (Henderson et al. 1984; Wiegand 1996). In addition, docosahexaenoic acid (DHA, 22:6n-3) has been shown to be a poor substrate for mitochondrial β-oxidation (Madsen et al. 1998; Tocher 2015, p. 200). Contrary to the general notion that marine fishes lack the ability to biosynthesize HUFA (Sargent et al. 1993, 2002), recent findings suggest that HUFA biosynthetic capability varies considerably among species (Monroig et al. 2018). Some marine fishes may be able to convert 18:2n-6 and 18:3n-3 into longer chain FA (e.g., 20:3n-6; 20:4n-3, respectively; Figure 2.5; Monroig et al. 2018). However, biosynthesis of the most abundant and physiologically important HUFA (e.g. ARA, eicosapentaenoic acid [EPA, 20:5n-3] and DHA) is inhibited by the lack of functional enzymes in some species, such as Nibe croaker (*Nibea mitsukurii*), which is closely related to red drum (Figure 2.5; Kabeya et al. 2015; Castro et al. 2016). As such, the transfer of FA from the diet to the oocyte is expected to vary substantially among FA.

Our findings suggest little modification of PUFA between ingestion and deposition in eggs by red drum, as evidenced by the consistent and strong correlations between diet and egg for all (n-3) and (n-6) PUFA except 18:3n-6, 20:2n-6 and 22:5n-3 (Figure 2.3). Being intermediary products between 18:2n-6 and 20:3n-6 (Figure 2.5; Castro et al. 2016; Monroig et al. 2018), the dietary signals of 18:3n-6 and 20:2n-6 in eggs may be obscured by biosynthesis, resulting in the lack of significant diet-egg correlations. The poor diet-egg correlation for 22:5n-3 may be the result of its oxidation for energy or elongation of EPA, both of which have been demonstrated in other species. Capelin (*Mallotus villosus*)

selectively mobilized and catabolized 22:5n-3 during reproduction (Henderson et al. 1984). Nibe croaker can elongate EPA into 22:5n-3 and then into 24:5n-3 (Figure 2.5; Kabeya et al. 2015).

Egg 16:2n-4 level showed close correspondence with dietary intake in our study. This FA is synthesized from 16:1n-7 by certain diatoms (Volkman et al. 1989; Dunstan et al. 1993) and is metabolically inert in consumers (Figure 2.5; Iverson 2009). Some animals (e.g., rats (Nakanon et al. 1999)) can convert 16:2n-4 to 16:3n-4 and 18:3n-4, but it is not known whether red drum are capable of this conversion. If this biosynthetic pathway is active, that could explain the lack of diet-egg correlations for 16:3n-4 and 18:3n-4. It is important to note that the lack of diet-egg correlations for 16:3n-4, 18:3n-4 and 20:2n-6, which were present in low concentrations, may simply be due to the insufficient variation in dietary intake (ΔFA) in our experiments (Table 2.1).

Synthesis or selective mobilization and oxidation might explain the poor diet-egg correlations for 16:0, 16:1n-7, 18:1n-7, 18:1n-9, ΣSFA and ΣMUFA in our study. *De novo* synthesis is likely to result in an increased amount of 16:0, the major product of *de novo* synthesis, in marine predators (Budge et al. 2006). Furthermore, SFA and MUFA are common energy substrates in fishes (Sargent et al. 2002; Dalsgaard and St. John 2004) and, as such, are mobilized and selectively oxidized for energy during reproduction (Henderson et al. 1984; Harel et al. 1994; Huynh et al. 2007). In addition, our analytical method did not measure fatty alcohols in wax esters. The oil globules in fish eggs contain high levels of wax esters (Wiegand 1996), which are synthesized in the ovary by reducing FA, primarily SFA and MUFA (e.g., > 90% of fatty alcohol in European sea bass (*Dicentrarchus labrax*) eggs (Bell et al. 1997)), to their corresponding fatty alcohol (Wiegand 1996). Conversion of dietary SFA and MUFA into fatty alcohols would have reduced our ability to detect diet-egg correlations.

Odd-chain FA (15:0, 17:0) are synthesized primarily by bacteria, therefore their sum has been used as a biomarker for bacterial production (Budge and Parrish 1998; Budge et al. 2001). However, this interpretation may apply best to detrital consumers (Iverson 2009). Higher trophic levels may derive odd-chain FA through synthesis by their gut flora (Budge et al. 2006). In our study, these FA were present in low concentrations in diets and eggs and did not show strong egg-diet correlations.

In addition to individual FA, we examined diet-egg relationships of FA sums and ratios that are commonly used as biomarkers of primary producers. Three ratios – 16:1n-7/16:0, ΣC16/ΣC18, and EPA/DHA – have been used, mostly in herbivores, to distinguish diets derived from diatom-based food webs *versus* from dinoflagellate-based food webs (Claustre et al. 1989; Budge and Parrish 1998). Laboratory experiments have verified that 16:1n-7/16:0 transfers from phytoplankton through copepods to Atlantic cod (*Gadus morhua*) larvae (St. John and Lund 1996). In our study, 16:1n-7/16:0 in eggs seemed to reflect dietary intake even though the components of this ratio did not (Figure 2.3). Diet-egg transfer of ΣC16/ΣC18 was not strong, which is consistent with the poor diet-egg relationships for the major components of these sums (16:0, 16:1n-7 and 18:1n-9). Although EPA and DHA in eggs appeared to be useful dietary markers separately, this was not true for their ratio. The ratios Σ(n-3)/Σ(n-6) for PUFA and HUFA have been used as an index of relative contribution of marine *versus* freshwater food sources (Henderson and Tocher 1987). In our study, these ratios and their individual sums had strong diet-egg correlations.

Fatty acids vs. lipids

We measured bulk FA in the samples, without separately measuring FA in the neutral lipid (NL) and polar lipid (PL) fractions. It has been found, however, that the FA

profile of a diet has a greater effect on FA composition of NL than that of PL in eggs (Wiegand 1996; Almansa et al. 1999; Furuita et al. 2002; Johnson 2009). Fish eggs that contain higher amounts of NL (those with oil globules; e.g., red drum) are more likely to be affected by dietary FA than those with higher amounts of PL (those without oil globules; e.g., cod) (Johnson 2009). Therefore, FA composition of eggs from species with higher PL content may be less sensitive to, and less informative of maternal diet. Future research should examine whether FA analysis of the NL fraction alone would improve the use of FA as trophic biomarkers (Iverson 2009), particularly for eggs with higher PL content.

Reproductive strategy

Bony fishes are generally either batch spawners, which produce multiple batches of eggs during a spawning season, or total spawners, which produce eggs during a single or very few spawns (McBride et al. 2015). Our study species, red drum, is a batch-spawning fish that produces millions of eggs during numerous spawning events within a reproductive season and, as a result, relies heavily on recent diet for nutrients that are deposited in eggs. In another batch-spawning fish, gilthead seabream (*Sparus aurata*), levels of 18:2n-6, EPA, and DHA in eggs equilibrated with dietary composition by 15 days after a diet shift (Harel et al. 1994). In contrast, fishes that decrease or cease feeding during ovarian maturation and spawning generally mobilize lipids from carcass, viscera, abdominal fat, muscle, and/or liver (Fernández-Palacios et al. 2011). Capelin mobilize lipids from muscle prior to spawning, with approximately 38% of the mobilized lipids transferred to the ovary and the remainder to support the energetic cost of reproduction (Henderson et al. 1984). It is important to note that diet could still influence egg FA composition of total spawners, but it may require a longer and earlier feeding period. For example, 4 months of feeding during

vitellogenesis altered the egg FA profiles of rainbow trout (*Oncorhynchus mykiss*) (Vassallo-Agius et al. 2001a) and coho salmon (*O. kisutch*) (Johnson et al. 2011).

Temporal dynamics and food web dynamics

Clarifying the temporal dynamics of diet-tissue transfer provides information about the time frame over which a tissue integrates a dietary signal. In contrast to body tissues that integrate dietary information over 7 to 8 weeks (e.g., muscle and liver) (Copeman et al. 2013; Mohan et al. 2016), FA incorporation in red drum eggs lags ingestion by 2.5 to 17 days (Figure 2.3). In addition, incorporation rate is proportional to the magnitude of change in dietary intake for 11 FA (Table 2.1), consistent with the simple dilution model (Robin et al. 2003; Jobling 2004). We did not observe equilibration of eggs to diet for about a month; egg FA level maintained a linear trend for one month after a diet shift (Figure 2.1). We caution that the lag for incorporation and the lack of equilibration should be taken into consideration when FATM and QFASA are applied to study adult diet composition and its temporal and spatial variations.

Results of our study demonstrate the importance of understanding the dynamics of diet-egg transfer of individual FA when examining the contribution of maternal diet to the variation of FA availability in egg boons. Large shifts in diet might occur naturally, for instance, when adults migrate to spawning areas that have a different prey field or when prey communities vary over time in response to ecological disturbance (e.g., drought, cyclone, marine heat wave, regime shift). Once the major consumers of eggs have been identified, the knowledge of diet-egg transfer dynamics of FA provided here can be used to clarify the trophic linkages that are mediated by egg boons. We expect FA in eggs will be useful for inferring adult diets for other species that exhibit similar reproductive

characteristics and for understanding counter-gradient trophic transfer, from adult fish to lower trophic levels, via egg boons.

Table 2.1: Summary of regression of I_{FA} on ΔFA for each FA.

Minimum and maximum ΔFA show the range of diet change in the analysis for each FA. Boldface type indicates FA having a significant linear relationship between I_{FA} and ΔFA. R^2 is the coefficient of determination and p is statistical significance. Minimum and maximum ΔFA for ratios are dimensionless.

Fatty acid	Minimum and maximum ΔFA (mg wk^{-1})	Slope	R^2	p
Saturated fatty acids				
14:0	-388, 955	$9.00e^{-5}$	0.21	<0.05
15:0	-543, 471		0.11	0.16
16:0	-2171, 5883		0.03	0.50
17:0	-541, 467		0.16	0.07
18:0	-1876, 1891	$3.74e^{-5}$	0.27	<0.05
ΣSAT	-3385, 7516		0.00	0.85
Monounsaturated fatty acids				
16:1n-7	-435, 846		0.09	0.18
18:1n-7	-430, 1135		0.05	0.35
18:1n-9	-508, 3412		0.01	0.67
20:1n-9	-596, 1284	$2.30e^{-5}$	0.30	<0.05
22:1n-11	-314, 685		0.04	0.41
ΣMUFA	-1317, 20190		0.02	0.52
Polyunsaturated fatty acids				
n-4 fatty acids				
16:2n-4	-120, 259	$1.43e^{-4}$	0.24	<0.05
16:3n-4	-64, 145	$2.26e^{-4}$	0.19	<0.05
18:3n-4	-23, 54		0.00	0.96
n-6 fatty acids				
18:2n-6	-835, 1011	$1.25e^{-4}$	0.65	<0.01
18:3n-6	-215, 175		0.00	0.80
20:2n-6	-32, 85		0.10	0.18

Table 2.1
(continued)

Fatty acid	Minimum and maximum ΔFA (mg wk^{-1})	Slope	R^2	p
20:3n-6	-174, 61	$9.78e^{-5}$	0.40	<0.01
20:4n-6 (ARA)	-441, 473	$1.56e^{-4}$	0.37	<0.01
22:4n-6	-84, 61		0.16	0.06
22:5n-6	-153, 147		0.15	0.08
Σ(n-6) PUFA	-1137, 916	$1.77e^{-4}$	0.55	<0.01
Σ(n-6) HUFA	-583, 548	$2.00e^{-4}$	0.45	<0.01
n-3 fatty acids				
18:3n-3	-103, 249	$9.78e^{-5}$	0.23	<0.05
18:4n-3	-148, 339		0.18	0.06
20:3n-3	-68, 153	$7.31e^{-5}$	0.36	<0.01
20:4n-3	-113, 254		0.06	0.28
20:5n-3 (EPA)	-3057, 6729		0.14	0.10
22:5n-3	-263, 292		0.07	0.26
22:6n-3 (DHA)	-3575, 7969	$8.01e^{-5}$	0.31	<0.05
Σ(n-3) PUFA	-6840, 15784	$5.10e^{-5}$	0.20	<0.05
Σ(n-3) HUFA	-6621, 15262	$5.21e^{-5}$	0.21	<0.05
Other sums and ratios				
16:1n7/16:0	-1, 1		0.00	0.86
ΣC16/ΣC18	-3, 4		0.11	0.16
DHA/EPA	-14, 8		0.10	0.17
ΣPUFA	-6466, 15462		0.12	0.14
ΣHUFA	-6460, 15110		0.14	0.10
Σ(n-3)/Σ(n-6) PUFA	-32, 51	$4.18e^{-3}$	0.69	<0.01
Σ(n-3)/Σ(n-6) HUFA	-45, 42	$4.54e^{-3}$	0.74	<0.01

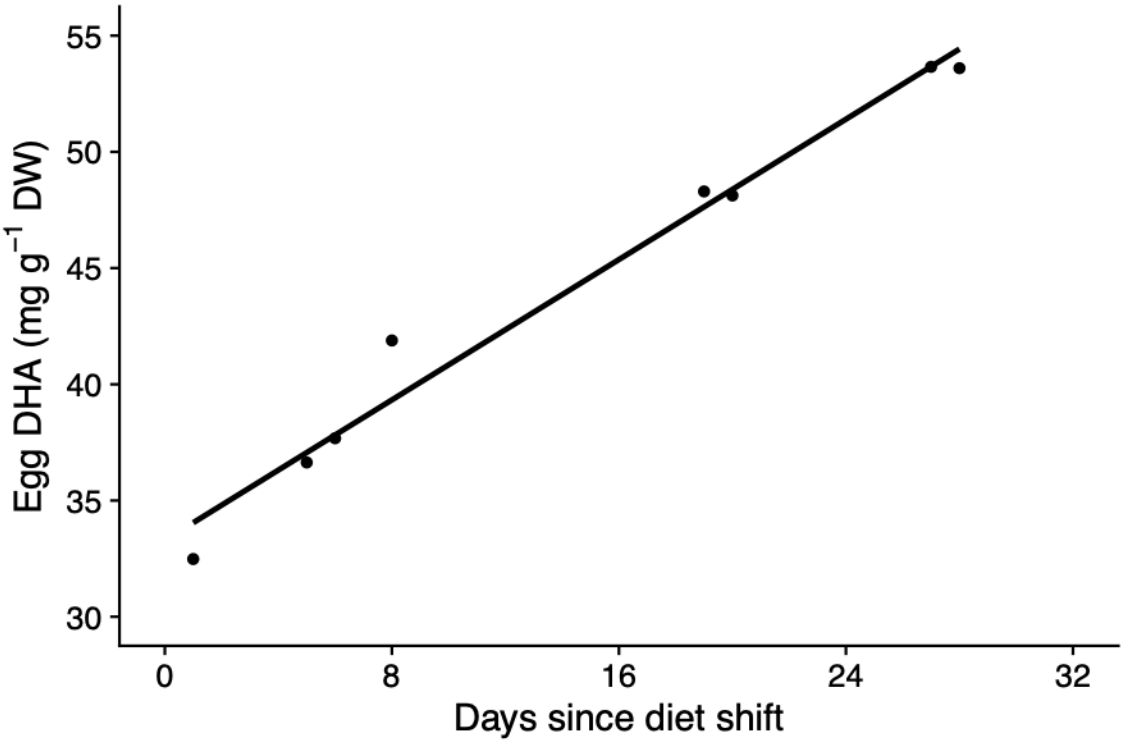

Figure 2.1: An example of incorporation rate estimation.

Representative time course for incorporation of DHA into eggs after a diet shift of ΔDHA = 3018 mg wk^{-1} (Experiment 8). Regression slope indicates I_{DHA} = 0.75 mg g-1 DW d^{-1}.

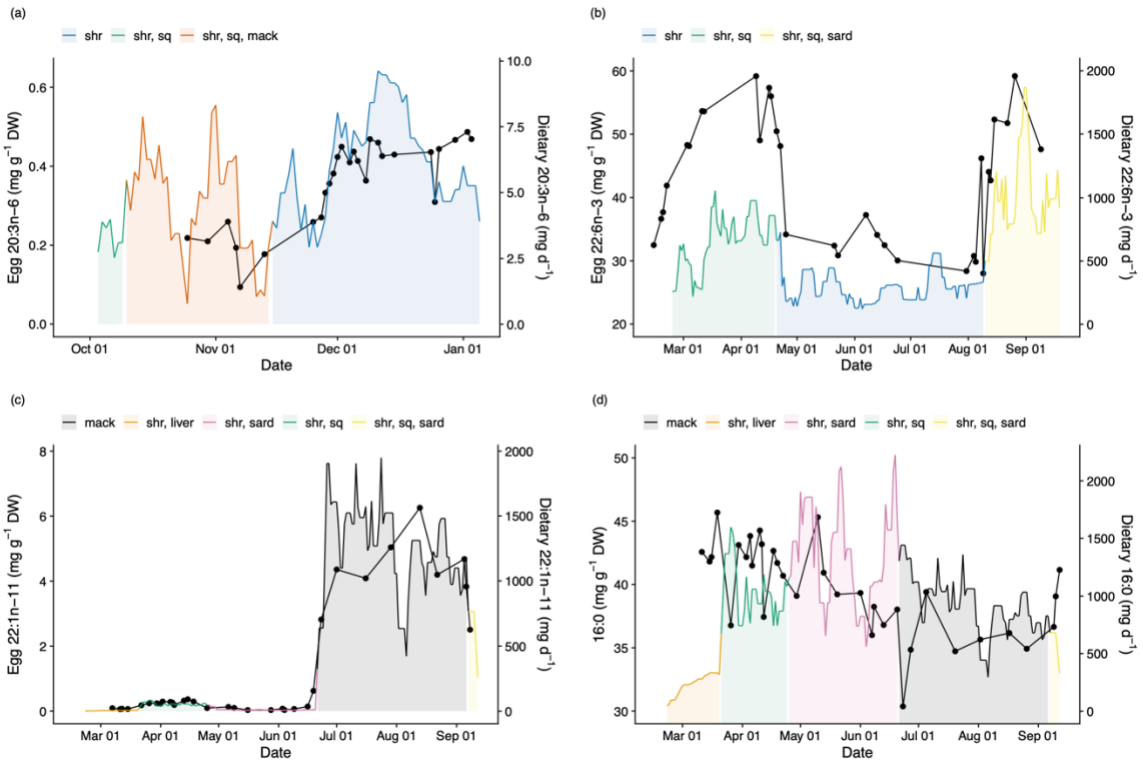

Figure 2.2: Examples of lag analysis.

Egg FA content (circles) and 7-day moving average of daily dietary intake (shaded area). (a-c) Examples of significant diet-egg correlations. To highlight correlations, egg data were shifted to the left by the calculated lags of (a) 2 days (maximum r = 0.67) for 20:3n-6 in MT8; (b) 10 days (maximum r = 0.78) for DHA (22:6n-3) in MT9; (c) 4 days (maximum r = 0.97) for 22:1n-11 in MT1. (d) Example of absence of diet-egg correlation (maximum r = -0.09) for 16:0 in MT1. Colors indicate periods of different adult diets. Diet items include mackerel (mack), shrimp (shr), sardine (sard), squid (sq) and beef liver (liver).

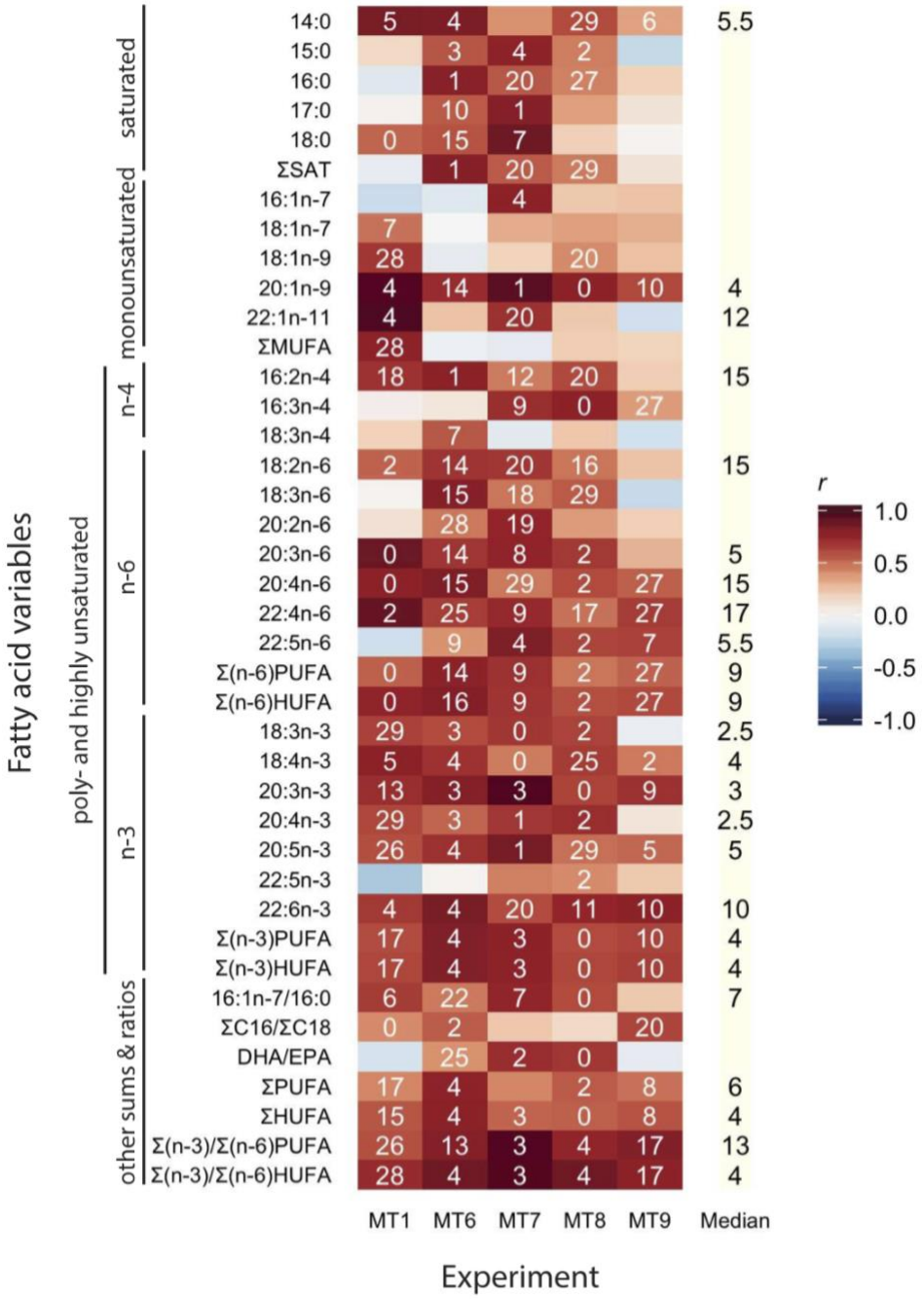

Figure 2.3: Heat map showing results of lag analysis.

Color of each cell indicates the maximum correlation (*r*) between egg FA content and daily dietary intake for lags of 0-30 days. Columns identify separate multiple-diet-shift experiments. Value in each cell is the lag (in days) corresponding to the maximum *r*. Median lags are shown for FA in which correlations were significant for at least 4 of the experiments.

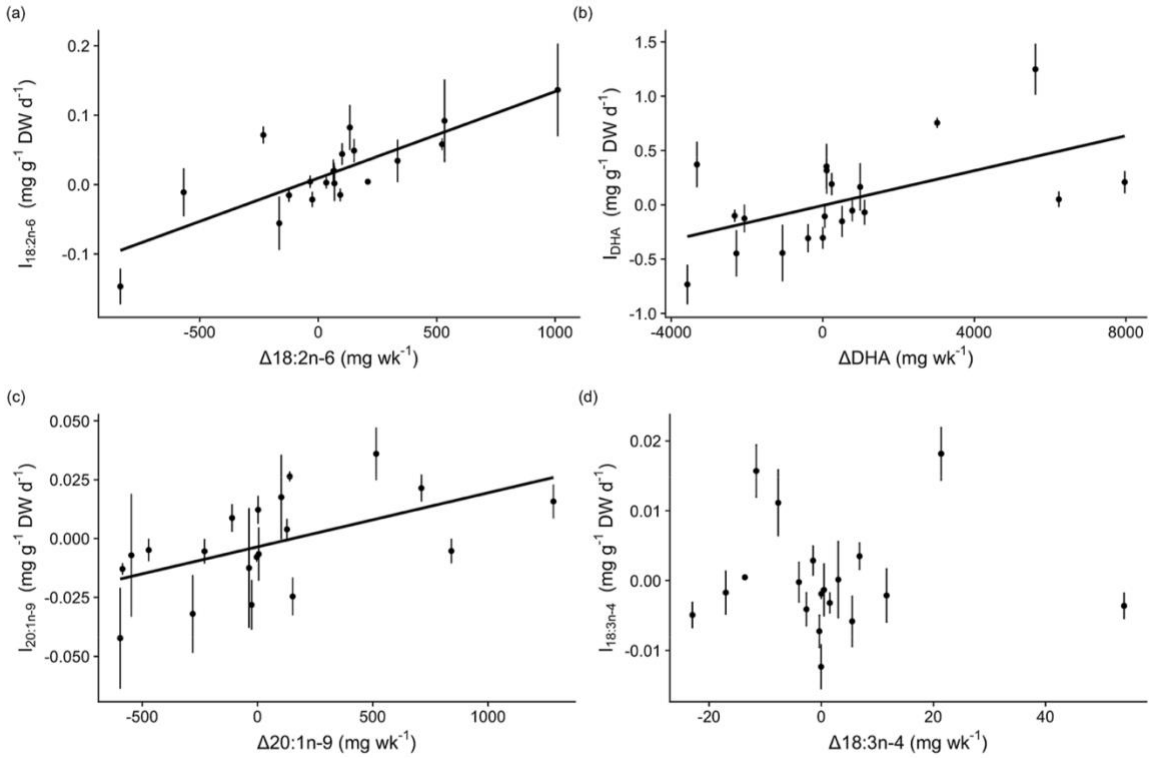

Figure 2.4: Examples of incorporation rate analysis.

There was a significant linear relationship between the rate of incorporation of FA into eggs (I_{FA}) and the magnitude of change in dietary intake (ΔFA) for (a) 18:2n-6 (R^2 = 0.65, $p < 0.05$); (b) DHA (22:6n-3; $R^2 = 0.31$, $p < 0.05$); (c) 20:1n-9 ($R^2 = 0.30$, $p < 0.05$); (d) but not for 18:3n-4 ($R^2 < 0.01$, $p = 0.96$). Each I_{FA} value was estimated as the slope of a linear regression fitted to the time course of a single-diet-shift experiment (Figure 2.1). Error bars are ±1 s.e. of the slope. Complete results are in Table 2.1.

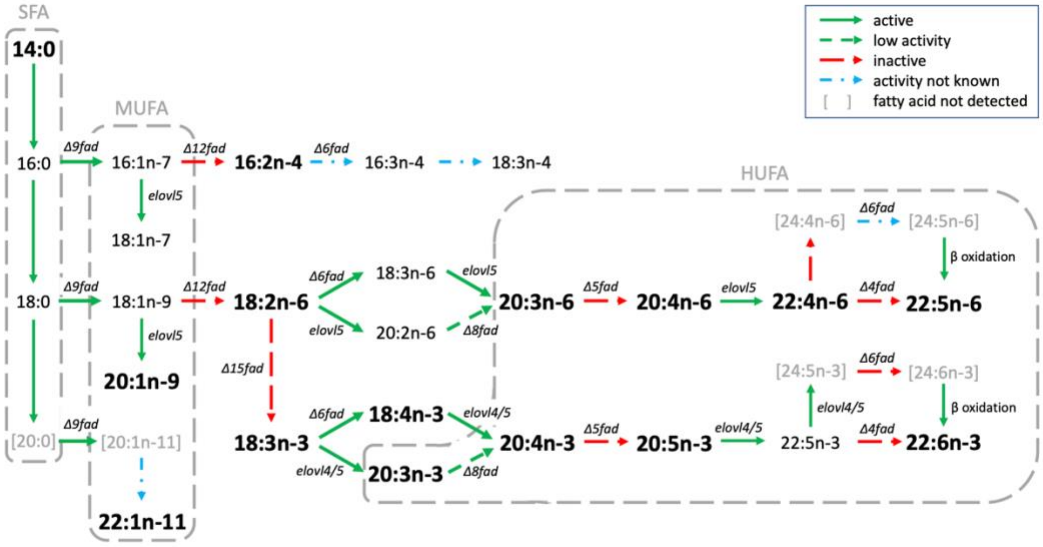

Figure 2.5: Putative FA biosynthetic pathway for red drum.

Arrows show level of activity for each step, based on findings for a closely related species, Nibe croaker (Kabeya et al. 2015). FA in boldface type are those deemed useful trophic markers in red drum eggs, based on results from the current study. Desaturation reactions are denoted by Δn, where n indicates the carbon position at which a double bond is inserted. Elongation reactions are catalyzed by enzymes encoded by genes shown in italics.

Chapter 3: Incorporation of Dietary Lipids and Fatty Acids into Red Drum *Sciaenops ocellatus* Eggs

INTRODUCTION

Eggs contain the only source of nutrients available to the developing embryos and larvae of oviparous fishes before the onset of exogenous feeding. Maternal provisioning of nutrients can have a critical influence on the growth and development of the offspring (Rainuzzo et al. 1997; Izquierdo et al. 2001). The composition of these maternally derived nutrients, however, partly depends on maternal diet (Wiegand 1996; Johnson 2009; Zakeri et al. 2014; Hou et al. 2020). Maternal diet-induced variability in egg fatty acids is well described in many fish species, but little is known about the influence of dietary lipids on egg composition. Such knowledge is important for understanding the process of nutrient transfer from adults to embryos and provides more insight into the connection between maternal nutrition and offspring metabolism and performance.

Female fishes incorporate nutrients into developing oocytes from their dietary intake, body stores, or *de novo* synthesis (Wiegand 1996; Johnson 2009). The relative contribution of each of these sources is related to the species' reproductive strategy. For semelparous species that decrease or stop feed intake prior to or during spawning, the energy and materials needed for oocyte development may derive from body stores, such as abdominal fat, muscle, and liver. For iteroparous species that continue to feed through sexual maturation and spawning, nutrients that are deposited in the eggs may be largely derived from the recent diet (Johnson 2009; Fernández-Palacios et al. 2011). These conditions largely align with the concepts of capital and income breeding, which describe the two extremes of a spectrum of how much an organism relies on previously acquired (stored) versus current resources (Jönsson 1997; McBride et al. 2015).

Lipids and constituent fatty acids serve key energetic and structural roles for marine fish embryos and larvae (Heming and Buddington 1988; Rainuzzo et al. 1997). Generally, neutral lipids (NL) function as the main energy source after hatching, which sustains larvae during the period of rapid growth and development and transition to exogenous nutrition (Sargent et al. 2002; Kamler 2008). Polar lipids (PL) are essential components of cell membranes, which are synthesized during this period of intense cell proliferation and differentiation. Polar lipids also are a source of phosphate for nucleic acid synthesis and choline for neurotransmission (Rainuzzo et al. 1997). Some fish species that produce eggs with a high polar lipid content also catabolize polar lipids for energy during embryonic and early larval development (e.g., Atlantic herring *Clupea harengus*, Atlantic cod *gadus morhua,* Atlantic halibut *Hippoglossus hippoglossus*; Rainuzzo et al. 1997; Sargent et al. 2002; Tocher et al. 2008).

Egg fatty acid composition is closely related to spawning success, egg quality, and subsequent larval growth and development (Rainuzzo et al. 1997; Izquierdo et al. 2001; Sargent et al. 2002). As a result, tremendous efforts have been made to understand diet-induced variability in egg compositions. In recent years, mounting evidence has shown that changes in maternal diets can alter egg fatty acid composition (Mourente and Odriozola 1990; Harel et al. 1994; Almansa et al. 1999; Furuita et al. 2002; Li et al. 2005; Fuiman and Faulk 2013; Hou et al. 2020) and lead to both short-term and long-term effects on larval metabolism and performance (Fuiman and Ojanguren 2011; Morais et al. 2014; Perez and Fuiman 2015; Burns and Fuiman 2019). However, in direct contrast to the greater research interest focusing on egg fatty acids, there is a lack of information regarding the influence of diet on lipid composition of eggs. Lipid molecules, as a whole, are metabolized differently due to their diverse structures and functions, which might affect the fate of their constituent fatty acids during various biological processes (e.g., digestion,

transport, absorption, lipid accumulation during oogenesis, organogenesis, and energy metabolism). Therefore, when examining only fatty acid profiles in egg total lipids, important information may be lost regarding lipid metabolism in both the adult females and the offspring.

We have previously conducted a series of diet-shift experiments on groups of spawning red drum *Sciaenops ocellatus* and found that concentrations of 15 fatty acids (out of 27 measured) in eggs were positively correlated with their levels in the diet, and that changes could be detected in eggs within 2.5-17 days of a diet shift (Hou et al. 2020). The present study provides a more complete assessment of how maternal diet affects egg lipid composition by addressing whether the dietary effects on egg fatty acid composition pertain to particular lipid classes. This knowledge may provide insight into the process of maternal-offspring nutrient transfer, the important roles of maternally-derived lipids during early growth and development, and the consequences of variations in maternal diets to offspring physiology and performance.

METHODS AND MATERIALS

Broodstock care and diet treatments

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin (AUP-2016-00011 and AUP-2018-00302). Adult red drum *Sciaenops ocellatus* (86-96 cm length, 7.6-11.1 kg weight, age undetermined) were captured in nearby waters at least 1 year prior to the experiment and were maintained at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute in Port Aransas, TX. They were held in 12,000 or 16,000 L recirculating tanks at a controlled temperature (24-28°C), salinity (30-36 ppt), photoperiod (11:13 L:D), and 6.15-7.40 mg L^{-1} dissolved oxygen. Fish were induced to spawn naturally

by manipulating temperature and photoperiod (a gradual shift from 14:10 L:D to 10:14 L:D regime; Arnold et al. 1977).

Diet-shift studies were conducted following previous studies (Fuiman and Faulk 2013; Hou et al. 2020). Adult red drum were fed one of six diets 3-5 times per week until satiation: (1) Atlantic thread herring *Opisthonema oglinum*, (2) beef liver & squid *Loligo opalescens* (equal wet weight of squid were added to facilitate spawning), (3) Atlantic mackerel *Scomber scombrus*, (4) Spanish sardine *Sardinella aurita*, (5) shrimp *Litopenaeus setiferus*, (6) squid. Diet items were previous frozen and thawed before feeding. The amount of ingested food per meal was recorded.

Sample collection

Spawning occurred at about 2000 hours every 2-10 days on average. Fertilized eggs were collected at 0800 hours the following morning. Approximately 500 eggs were rinsed with distilled water and frozen at -80 °C until analysis. Previous studies have shown that diet shifts change fatty acid composition in the eggs within 2-17 days (Fuiman and Faulk 2013; Hou et al. 2020). All spawns after the diet shifts were collected for fatty acid analysis to confirm that the fatty acid composition (and potentially lipid composition) of the eggs had responded to the diet change. Two spawns that were produced 2 and 3 days after the diet shifts and had not yet reflected dietary intake were excluded; the rest of the spawns (≥ 5 days after diet shifts) were retained for further analysis (n = 5 to 9 spawns for each diet group; Table 3.1). Eggs from two broodstock tanks that were both fed the sardine diet (Table 3.1) were combined because t-tests showed no significant differences in any egg lipid class concentrations, indicating no significant female effect.

Three to eight samples of each diet item were collected during the diet-shift studies for fatty acid and lipid class analyses. Whole animals were homogenized and each sample

was separated into 2-4 subsamples (depending on sizes of sample items) and frozen at -80 °C until analysis. Both dry weight (dw) and wet weight were recorded (Table B1). For each sample of a diet item, lipids were extracted from 3-5 subsamples (dw = approximately 20-100 mg, depending on lipid content) and analyzed for fatty acid profile (in total lipids), and 2-4 subsamples (dw = approximately 30-300 mg) were analyzed for lipid class composition and fatty acid profiles in major lipid classes.

Biochemical analysis

Fatty acids in total lipids of diet items and eggs were methylated and measured by gas chromatography (GC) following established methods (Faulk and Holt 2005). Briefly, lyophilized and homogenized samples were extracted with 2:1 chloroform: methanol (v/v) following the method by Folch et al. (1957). A known amount of tricosanoic acid (23:0; Supelco, Inc.) was added before homogenization as an internal standard. Fatty acid methyl esters (FAME) were prepared by saponification in potassium hydroxide in methanol, followed by transesterification with 14% boron trifluoride in methanol. FAME were dissolved in 500 µl hexane before analysis by GC-2014 (Shimadzu Scientific Instruments) using a flame ionization detector and a Supelcowax 10 column (30 m long, 0.53 mm internal diameter, 1.0 um thickness; Supelco, Inc.).

A second lipid extraction using the same method was performed (without the internal standard) for lipid class analysis. The extracted lipids were evaporated under a stream of nitrogen, and, with the lipid weight determined gravimetrically, were dissolved in a known concentration (20 mg ml^{-1}) of 2:1 chloroform:methanol with 50 mg L^{-1} of butylated hydroxytoluene (BHT) as an antioxidant. Lipid classes were separated and quantified by ultra-high performance liquid chromatography (UHPLC) using methods detailed below.

1. Chemicals and reagents

Isooctane, ethyl acetate, acetone, diethylamine, formic acid, isoproponal, methanol, 14% boron trifluoride, and chloroform (ACS, LC/MS or HPLC grade) were purchased from Fisher Scientific or Millipore Sigma. Standards for lipid classes of interest included: phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylcholine (PC), sphingomyelin (SM), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), phosphatidylserine (PS), cardiolipins (CL), phosphorylglycerol (PG (12:0/12:0) and PG (16:0/18:1)), wax ester (WE, cetyl palmitate), steryl ester (SE, cholesteryl linoleate), free fatty acid (FFA, stearic acid), triglyceride (TG, glyceryl trioleate), sterol (ST, cholesterol), diacylglycerols (DG (18:0/18:0) and DG (18:1/18:1)), monoacylglycerol (MG (18:0)). Lipid standards were purchased from Millipore Sigma, Matreya LLC, Cayman Chemical, or Avanti Polar Lipids Inc, and were the highest purity available (≥ 95%).

2. Apparatus

The UHPLC instrumentation consisted of a Thermo Scientific LPG3400-SD pump, WPS-3000 FC autosampler with fraction collector, TCC-3000 column oven, and Corona ultra RS Veo Charged Aerosol Detector (CAD) equipped with a Syncronis Silica 5 μm, 2.1 x 150 mm column (Thermo Scientific) and a nitrogen generator and air compressor (Peak Scientific). Separation of lipids was performed with a column temperature of 40 °C. The CAD was set at a gain of 16, 10 s filter, an air pressure of 35 psi and a temperature of 35 °C.

3. Chromatographic methods

Lipid profiles were quantified using separate normal-phase applications for neutral and polar lipids. Neutral lipids (WE, SE, TG, DG, MG, FFA, ST) were characterized following methods developed by Abreu et al. (2017) with modifications. The mobile phase

and the solvent program are shown in Table B2. Since marine fish eggs and some diet items contain high concentrations of neutral lipids and low concentrations of polar lipids, a second program is often needed for accurate polar lipid quantification (Silversand and Haux 1997). Polar lipids (PE, PG, PI, PA, PS, CL, PC, SM, LPC) were characterized using methods adapted from Plante et al. (2016). The mobile phase and the solvent program are shown in Table B3.

4. Quantification

Lipid standards were injected individually to confirm retention times and peak shapes. They were then mixed together and injected to assess coelution. Most standards (PE, PC, SM, LPC, PG, FFA, TG, ST, DG, MG) eluted as single peaks. Pairs of standards coeluted or overlapped significantly (WE and SE, PI and PA, PS and CL). Given the known trace amounts or absence of PA and CL in fish egg samples (Silversand and Haux 1997), only PI and PS standards were used during quantification, respectively. The WE and SE peak was identified as WE/SE and equal amounts of WE and SE standards were used in the standard mix. DG and MG were not detected in quantifiable amounts in any of the samples.

Standards for lipid classes of interest were mixed in known concentrations and were prepared separately for neutral lipids and polar lipids (standard mix compositions varied according to sample type). A 4- to 6-point calibration curve was generated for each lipid class by injecting different volumes of the prepared standard mix solutions, with each volume injected repeatedly 2-3 times, interspersed during sample runs. The coefficient of determination (R^2) was used to assess the goodness of fit for each calibration curve. Power function transformation was applied to CAD output during neutral lipid quantification to improve linear range and reduce peak width and asymmetry (Crafts et al. 2016). The power

function value (PFV) varied depending on the chemistry of liquid phase and mass range of the analyte (Crafts et al. 2016). Therefore, a calibration curve for each neutral lipid was produced at a PFV of 1.1-1.7 (0.1 increments), and the PFV that returned the greatest R^2 (> 0.99) was determined as optimal. Each neutral lipid class was quantified based on the calibration curve at its optimal PFV. For polar lipid quantification, calibration curves were expressed as a second order polynomial function ($R^2 > 0.99$).

5. Fraction collection

Major lipid classes WE/SE, TG, PE, PC, FFA (when approximately > 5% in samples, which varied according to sample types) were collected following the column separation on UHPLC and their fatty acid profiles were quantified using GC, as fatty acids from these major lipid classes would represent the majority of fatty acids in the total lipids. Fatty acids from minor lipid classes (<5% total lipids) could not be measured reliably because they were below the limit of quantitation for GC. Collected fractions were dried under a nitrogen stream and methylated using the method described above. FAME were dissolved in 100 µl□hexane. An injection volume of 8 µl and a split ratio of 2:1 was used. To evaluate the potential influence of UHPLC solvents on GC analysis, fractions were collected for two blank runs (with no injection) during the same retention time as each lipid fraction, which were then methylated and analyzed on GC. No significant peaks from UHPLC solvents were detected.

Data analysis

Lipid class concentrations were quantified as mg g^{-1} dw or % total lipids (total lipids were determined gravimetrically as previously mentioned). Fatty acids were quantified as mg g^{-1} dw and % total fatty acids in total lipids, and as % total fatty acids in lipid classes.

The sums of groups of fatty acids were also calculated: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), highly unsaturated fatty acids (HUFA), n-3 HUFA, n-6 HUFA[4].

Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences in egg lipid composition (7 lipid classes) and egg fatty acid composition (27 fatty acids) in total lipids, WE/SE, TG, and PC among different diet groups, followed by *a posteriori* pairwise comparisons and one-way ANOVA and Tukey's tests (performed on each lipid class) when PERMANOVA result was significant. Multivariate homogeneity of group dispersions, normality, and homogeneity of variance were tested. The non-parametric Kruskal-Wallis test was used when normality was violated. The false discovery rate ($\alpha = 0.05$) was employed for multiple comparisons (Benjamini and Hochberg 1995).

Principal components analysis (PCA) was performed separately on the fatty acid profiles (% of total fatty acids) of (1) total lipids in diet items, (2) major lipid classes in diet items, (3) total lipids in eggs, (4) major lipid classes in eggs. One-way ANOVA was used to test for significant differences in PCA scores among different groups. Assumptions of normality and homogeneity of variance were tested. The Welch one-way ANOVA was used when homogeneity of variance was violated, followed by Games-Howell tests. For the PCA performed on (4) fatty acid profiles of major lipid classes in eggs, observations were separated into groups based on the lipid classes and the mean multidimensional distance (Euclidean distance) from each point to the group center was calculated, as a measure of dispersion (i.e., within-group variations).

[4] PUFA include FA with ≥ 2 double bonds. HUFA include fatty acids with ≥ 20 carbon atoms and ≥ 3 double bonds in our calculation.

Mean weekly dietary intake of individual fatty acids ($Intake_{FA}$; mg $fish^{-1}$ $week^{-1}$) by adult fish during the experimental periods was calculated as:

$$Intake_{FA} = \frac{WW \times DW \times [FA]}{n},$$

where, *WW* was the mean weekly wet weight of food (g) consumed by a broodstock tank, *DW* was the ratio of dry weight to wet weight, [*FA*] was the concentration of a fatty acid in the diet item (mg g^{-1} dw), and *n* was the number of fish in the tank (Table 3.1 & B1). Pearson's correlation coefficient (*r*) was calculated relating the egg fatty acid concentrations (% total fatty acids) in total lipids, WE/SE, TG, and PC to the mean weekly dietary intake of the same fatty acid. A positive and statistically significant (one-tailed test, $p < 0.05$) *r* indicated a strong dietary influence for a fatty acid in the eggs.

RESULTS

Diet lipid profiles

Among the six diet items, mackerel had the greatest amount of total lipid, followed by liver, whereas shrimp contained the lowest levels of total lipid (Figure 3.1a). Lipid profiles were different across diet items (Figure 3.1c; Table 3.2). Oily fish (mackerel, sardine, herring) were characterized by high levels of neutral lipids, which were predominantly TG (> 60% total lipid). Liver and shrimp were characterized by high levels of polar lipids (> 75% total lipid), predominantly PC. Squid consisted of approximately 2/3 neutral lipids and 1/3 polar lipids.

Egg lipid profiles

The major lipid classes in red drum eggs were WE/SE, TG, and PC, accounting for 79.0 ± 6.2% of total lipids (Table 3.3; 26.7 ± 4.1% WE/SE, 30.0 ± 5.0% TG, 22.6 ± 3.0% PC). ST, PE, PI/PA, and PS were present in low concentrations (Table 3.3; 3.8 ± 0.5%, 3.9

± 0.7 %, 2.1 ± 0.8%, 0.8 ± 0.2%, respectively; total lipids were measured gravimetrically after lipid extractions, and contained unidentified components). Lipid class composition of eggs was significantly different among the diet groups (Figure 3.1d; PERMANOVA *pseudo-*$F_{(5, 35)}$ = 3.8; P = 0.001). All three neutral lipid classes, WE/SE, TG, and ST, and one polar lipid class, PI/PA, were significantly different among diet groups (Table 3.3; ANOVA, FDR = 0.05).

Diet fatty acid profiles

The fatty acid composition in total lipids was different among diets (Figure 3.2a; Table B4). When fatty acids in the total lipids were summarized by principal components analysis, the first two principal component scores differed significantly among diet items (Welch ANOVA, $F_{(5, 47.9)}$ = 526.8, Welch ANOVA, $F_{(5, 47.4)}$ = 1310, respectively; P < 0.001). Liver was characterized by high 18:0 and n-6 PUFA content; squid, herring, and sardine contained high levels of 18:1n-7, 20:5n-3 (eicosapentaenoic acid, EPA), and 22:6n-3 (docosahexaenoic acid, DHA); and mackerel was characterized by high levels of 20:1n-9 and 22:1n-11 (Figure 3.2a; Table B4). Fatty acid profiles for sardine and herring were similar on the first two principal component scores.

Fatty acid profiles of major lipid classes showed patterns that were consistent with those of the total lipids for diet items (Figure 3.2a & 3.3), especially for the most abundant lipid class. For example, the differences in fatty acid composition between mackerel and the other diets were most prominent in TG (Figure 3.3), which was the most abundant lipid class in mackerel (Table 3.2). Similarly, differences between liver and all other diets were most prominent in PE and PC, which were the two most abundant lipid classes in liver.

Egg fatty acid profiles

Fatty acid profiles of total lipids in eggs were significantly different among diet groups (PERMANOVA *pseudo-*$F_{(5, 35)}$ = 43.4; P < 0.001), with all pairwise comparisons being significant (adjusted P < 0.05). Eggs from the mackerel diet were characterized by high levels of 14:0, 18:4n-3, 20:4n-3, 20:1n-9, 22:1n-11; and eggs from the liver & squid diet were characterized by high levels of 18:0 and n-6 PUFA (Figure 3.2b; Table B7). In addition, fatty acid profiles of WE/SE, TG, and PC were all significantly different among diet groups (Figure 3.4; PERMANOVA *pseudo-*$F_{(5, 35)}$ = 37.4, 19.5, 45.1, respectively; P < 0.001), with all the diet groups being significantly different from each other (adjusted P < 0.05) except for the sardine and the herring diet groups in WE/SE and PC (Figure 3.4a,c).

The extent to which maternal diets changed egg fatty acid profiles within each lipid class was different. Fatty acid profiles of WE/SE varied most among diets, followed by TG, then PC (Figure 3.4 & 3.5), as measured by the significantly different multidimensional distance to the group centers (mean = 4.81, 3.53, and 2.90 for WE/SE, TG, and PC, respectively; ANOVA $F_{(2, 120)}$ = 24.01, P < 0.001). Grouping fatty acids by their chain length and desaturation level, mean coefficients of variation (CV) for SFA, MUFA, n-3 HUFA, n-6 HUFA and HUFA (across diet groups) were greater in neutral lipids (WE/SE, TG) than in PC (Table 3.4). Between the two neutral lipid classes, CVs in WE/SE were greater than in TG for all except MUFA.

For some fatty acids (16:2n-4, 17:0, 18:2n-6, 20:1n-9, 20:3n-6, 20:4n-6 (arachidonic acid, ARA), EPA, 22:4n-6, 22:5n-6, DHA), correlations between parental dietary intake of the fatty acid (mg wk^{-1} $fish^{-1}$) and their proportional representation in eggs (% total fatty acids) were high for total lipids and all three lipid classes (Figure 3.6). For other fatty acids (18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 22:1n-11), diet-egg correlations were

strong in neutral lipid classes and total lipids, but lower in PC. For 14:0, 15:0, 18:0, diet-egg correlations were high in total lipid, TG, and PC, but not WE/SE (Figure 3.6).

There was a general pattern to the distribution of fatty acids among lipid classes that was not altered substantially by maternal diet. SFA were the highest in TG, followed by PC, and lowest in WE/SE (Figure 3.5; 43.5 ± 4.5% in TG, 38.5 ± 2.4% in PC, 14.2 ± 6.6% in WE/SE; ANOVA $F_{(2, 120)} = 430.6$, $P < 0.001$). MUFA were the highest in WE/SE, followed by TG, and lowest in PC (35.4 ± 7.3% in WE/SE, 26.8 ± 5.1% in TG, 13.5 ± 2.4% in PC; ANOVA $F_{(2, 120)} = 174$, $P < 0.001$). HUFA were the highest in PC, followed by WE/SE, and lowest in TG (37.7 ± 3.4% for PC, 31.8 ± 9.4% for WE/SE, 17.6 ± 4.6% for TG; ANOVA $F_{(2, 120)} = 108.7$, $P < 0.001$).

DISCUSSION

Dietary effect on egg lipid composition

Many previous studies have documented the effects of maternal diets on egg fatty acid composition, but relatively few have examined the effects on egg lipid class composition. While egg fatty acid profile can be greatly affected by maternal diet within a short period of time (Watanabe et al. 1984a; Mourente and Odriozola 1990; Harel et al. 1994; Navas et al. 1997; Bell et al. 1997; Lane and Kohler 2006; Lewis et al. 2011; Hou et al. 2020), the limited existing data suggest that egg lipid class profiles are relatively stable and independent of diet. For example, there were no changes in egg lipid class composition when adult gilthead seabream *Sparus aurata*, European seabass *Dicentrarchus labrax*, and rainbow trout *Oncorhynchus mykiss* were fed different diets (Mourente and Odriozola 1990; Bell et al. 1997; Vassallo-Agius et al. 2001). One study found that a fish oil diet provided during vitellogenesis (3 months before spawning) or all year induced lower levels of TG in European seabass eggs, compared with a maize oil diet during vitellogenesis

(Navas et al. 1997). In those studies, however, the fatty acid compositions of diets differed; the lipid class compositions of diets were either similar or not measured. In contrast, a few studies reported marked intraspecific variations in egg lipid class composition of fish eggs collected in the wild [e.g., 0.7-22% TG in bonefish *Albula vulpes* oocytes (Mejri et al. 2018); 40-86% phospholipids and 1.5-13% TG in Atlantic cod eggs (Bachan et al. 2012)] and in captivity [e.g., 40-70% PL and 10-44% TG in striped trumpeter *Latris lineata* eggs (Bransden et al. 2007)]. The causes of such variations were not well understood.

In red drum, there were no marked variations in egg lipid class compositions that mirrored the respective maternal diets, despite substantial differences in lipid class composition of those diets. There were, however, subtle differences in the concentrations of several lipid classes among diet groups. Those variations were greater for neutral lipid classes (WE/SE, TG, ST) than for polar lipid classes (PE, PI/PA, PS, PC). In particular, variations in egg TG content seemed to be driven by the TG content of the maternal diet. When TG levels in eggs (and diet) were low, levels of WE/SE in eggs increased, which resulted in relatively constant levels of neutral lipids in eggs for all diet groups. TG and WE/SE were the major constituents of neutral lipids in red drum eggs, accounting for approximately 45% and 50% of the neutral lipids, respectively. This inverse, compensatory change was also reported for European seabass eggs when egg composition varied in response to adult diets and interannually (Navas et al. 1997). Similar to our finding, phospholipids in eggs of European seabass and southern flounder *Paralichthys lethostigma* varied least over time or in response to maternal diet changes compared to other lipid classes (Navas et al. 1997; Mejri et al. 2021). These results suggest tight maternal control over both the structural components (phospholipids) and the energy substrates (neutral lipids) for the embryos. Overall, neutral lipids accounted for approximately 60% of the total crude lipid extracts and polar lipids accounted for 30%, which was similar to previous

results for red drum (Vetter et al. 1983) and comparable to other species' eggs with an oil globule, including common dentex *Dentex dentex*, gilthead seabream, pike *Esox lucius*, red seabream *Pagrus major*, sand eel *Ammodytes lancea*, turbot *Scophthalmus maximus*, and white seabream *Diplodus sargus* (Tocher and Sargent 1984; Watanabe et al. 1984a; Mourente and Odriozola 1990; Desvilettes et al. 1997; Mourente et al. 1999; Cejas et al. 2003; Cunha et al. 2013).

The majority of fatty acids, especially PUFA, transferred quickly from maternal diets to red drum eggs, which is consistent with previous findings (Fuiman and Faulk 2013; Hou et al. 2020). We further demonstrated that the dietary fatty acids were incorporated into all three major lipid classes (TG, WE/SE, PC), as indicated by the strong positive correlations between dietary intake levels and their proportional representation within each of the three lipid classes. In addition, diet-induced variations in fatty acid profiles, measured by the multidimensional distances and CVs for fatty acid groups, were greatest in WE/SE, followed by TG, and least in PC, suggesting stronger regulation and selection for incorporating fatty acids into PC than the neutral lipid classes. This is consistent with previous findings of a greater dietary effect on egg neutral lipid fatty acids than polar lipids fatty acids (gilthead seabream; Mourente and Odriozola 1990; Almansa et al. 1999; walleye *Sander vitreus*, Wiegand et al. 2004; white bass *Morone chrysops*, Lane and Kohler 2006; Lewis et al. 2011).

The different degrees of dietary influence on fatty acid compositions of neutral lipid and polar lipids may be partly explained by differences in the sources and pathways of lipid incorporation into oocytes. Dietary TG and phospholipids are hydrolyzed in the intestinal lumen (Olsen and Ringø 1997; Phan and Tso 2001; Tocher 2003). Upon uptake by enterocytes as bile salt micelles, the hydrolyzed products – MG, lysophospholipids and FFA – are reassembled as TG and phospholipids and predominantly form chylomicrons,

but also very low density lipoproteins (VLDL), which are exported from enterocytes to storage or utilization sites (Sheridan 1988; Tocher 2003). Plasma chylomicrons and VLDL contain mostly TG (85% and 52% of total weight, respectively) and their fatty acid compositions can be affected acutely after a meal (Tocher 2003). Lipids accumulated in the oocytes are primarily incorporated from two sources, VLDL and vitellogenin. Plasma VLDL, which are the primary source of oocyte neutral lipids, likely are sequestered by membrane receptor-mediated endocytosis or via the action of a lipoprotein lipase, which cleaves fatty acids from TG in the VLDL (Tocher 2003; Lubzens et al. 2010; Hiramatsu et al. 2012; Damsteegt et al. 2015; Reading et al. 2017). The acquired lipoproteins and fatty acids are then incorporated into lipid droplets in the previtellogenic oocytes (Johnson 2009). We speculate that the diet-derived VLDL are directly taken up by the developing oocytes after being exported from the enterocytes, and the chylomicrons are converted to VLDL in the liver with minimal modification of fatty acid compositions before being delivered to the ovary, resulting in the rapid changes in egg TG content and fatty acid composition observed following diet shifts. In contrast, fatty acid composition of vitellogenins, which are the main source of egg polar lipids and are synthesized in the liver, has been found to be strongly regulated and largely independent of liver fatty acid composition during oocyte maturation in some species (Silversand and Haux 1995). Consequently, eggs that contain high neutral lipids (oil globules) are generally more affected by diets than eggs that contain high polar lipids (Johnson 2009).

Indirect dietary effect on egg composition

Diets not only provide essential nutrients (i.e., those that cannot be synthesized by the females) for incorporation into eggs, such as HUFA, indispensable amino acids (e.g., taurine), and inorganic nutrients (e.g., phosphorus, choline), but also non-essential dietary

components, which may be used by females as precursors for synthesizing important molecules. For example, WE/SE accounted for around one third of red drum egg lipids but were barely detected in the diet items consumed by the adult fish. The significant positive correlations between fatty acid profiles of WE/SE and those of diets for 18 fatty acids suggest that the females used dietary fatty acids for WE/SE biosynthesis. Indeed, it has been shown that WE are present in low concentrations in female plasma or liver during reproduction despite its high concentration in the eggs (reviewed in Johnson 2009), and females synthesize WE in the ovary from fatty acid and fatty alcohol precursors (Sand et al. 1969; Wiegand 1996). Nevertheless, WE are abundant in some marine zooplankton from high latitudes (calanoid copepods, euphausiids), which are major prey for some marine fishes (Sargent et al. 2002; Tocher 2003; Tocher and Glencross 2015), although it is converted into TG during digestion and absorption by zooplanktivorous fish (Wiegand 1996; Sargent et al. 2002; Tocher 2003), and therefore, still has to be synthesized in the ovary of reproductive females.

Body stores can be an important source of nutrients (with a certain level of modification) for capital breeders, represented by migratory species such as capelin *Mallotus villosus*, bluefin tuna *Thunnus thynnus*, and Pacific herring *Clupea harengus pallasi* (Henderson et al. 1984; Mourente et al. 2002; Huynh et al. 2007) or species in the temperate or polar regions where food availability varies greatly seasonally, such as daubed shanny *Leptoclinus maculatus* (Murzina et al. 2012). On the other hand, the composition of body lipid stores is greatly affected or determined by diet (Hardy et al. 1987; Arzel et al. 1994; Bell et al. 2001, 2002; Garrido et al. 2008; Johnson et al. 2017). Therefore, there could be an indirect or delayed dietary effect on egg composition for capital spawners if an early and long duration of the experimental diet are considered, especially for species that mobilize body lipid stores to produce eggs with a high neutral lipid content (e.g.,

salmonids; Johnson 2009). Vitellogenesis appears to be the period when dietary lipids have a major impact on egg composition, but its time frame relative to spawning varies depending on a species' reproductive strategy (Rinchard and Kestemont 1996, 2003). Navas et al. (1997) showed that HUFA were incorporated most effectively into the developing oocytes during vitellogenesis in European seabass (~3 months prior to spawning). In rainbow trout, a diet given during vitellogenesis (4-6 months before spawning) had an effect on egg fatty acid profile (Vassallo-Agius et al. 2001b). In coho salmon *Oncorhynchus kisutch*, ^{13}C-labeled dietary lipids were also directly incorporated into ovarian lipids during secondary oocyte growth (~4 months prior to spawning; Johnson et al. 2011, 2017).

Some fishes adopt a mixed income-capital breeding strategy (McBride et al. 2015), as shown for Southern flounder, which mobilize stored nutrients to supplement the eggs when the diet is deficient in certain nutrients (Burns and Fuiman 2020; Mejri et al. 2021); and Iberian sardine *Sardina pilchardus*, which become more dependent on diets as the extended spawning depletes body reserves (Garrido et al. 2007). In batch spawners with prolonged spawning (short and continuous vitellogenic periods), such as gilthead seabream, a diet change during the spawning season can quickly produce measurable changes in egg composition and influence egg quality (Harel et al. 1994). But, such quick changes were not seen in a study done on the same species by Almansa et al. (1999), in which a diet deficient in n-3 HUFA fed 2 months prior to spawning did not change egg composition until 2 months after the onset of spawning. The authors suggested that maternal traits (weight and conditions) may be a factor. We suggest that the females may have drawn upon body stores to buffer against dietary deficiencies until the body reserves ran low.

Non-dietary effects on egg composition

Lipids ingested by adult females are often subjected to the influence of maternal metabolism ("consumer modification," Galloway and Budge 2020; Hou et al. 2020). The lack of diet-egg correlations for some fatty acids – 16:0 and several MUFA (16:1n-7, 18:1n-7, 18:1n-9) – was likely because females catabolized some dietary MUFA to meet energetic demands during spawning, since MUFA are the predominant energy sources for many fishes (Tocher 2003). Interestingly, long-chain MUFAs – 20:1n-9 and 22:1n-11 – are also considered metabolic energy sources (Tocher 2003), but their concentrations (in total lipids and all three fractions) in eggs were highly correlated with dietary intake (albeit proportionally lower in eggs compared to the diet). This discrepancy is likely due to greater modification of C16 and C18 MUFA than their C20 and C22 counterparts; either the C16 and C18 MUFAs were the preferred energy substrates for adult red drum during reproduction, or C16 and C18 MUFA were biosynthesized from the more abundant 16:0 and 18:0.

The general pattern of fatty acid composition within egg lipid classes was insensitive to maternal diet variations. The WE/SE fatty acid moiety in red drum eggs is characterized by low levels of SFA and high levels of MUFA and PUFA, which is consistent with some other studies (Bell et al. 1997; Lewis et al. 2011). The reverse is true for TG in eggs, which is characterized by a relatively high level of SFA and a low level of PUFA. High concentrations of PUFA and low concentrations of MUFA in polar lipids and high concentrations of MUFA in neutral lipids, as seen in our red drum eggs, have been reported in many fish eggs, regardless of their lipid class composition (e.g., white seabream with 2:1 NL:PL, or cod with 1:2 NL:PL Tocher and Sargent 1984; Watanabe et al. 1984b; Cejas et al. 2003; Lewis et al. 2011).

The variations observed in ST and PI/PA content of red drum eggs from different diet groups were likely not directly related to the dietary intake of these lipids, as their variations did not correlate positively with their respective content in the diet. Similarly, ST content of southern flounder eggs varied after diet shifts but was not correlated with dietary intake (Mejri et al. 2021). Teleosts, like other vertebrates, are able to biosynthesize cholesterol, the most abundant ST in animal tissues, and maintain its homeostasis in response to variations in dietary intake (Norambuena et al. 2013). In addition, cholesterol is not only an important component in cell membranes, but also a precursor of bile acid, steroid hormones, and vitamin D (Parish et al. 2008). The esterified form, cholesteryl ester (a major form of SE), is an important constituent of egg neutral lipids. Therefore, the measured concentration of ST in eggs may be a consequence of its involvement in several metabolic pathways. Similarly, both PI and PA are present in fish eggs in low abundance, as in most animal tissues, but are involved in many metabolic pathways. The derivatives of PI, inositol phosphates and DG, serve as intracellular second messengers (Wiegand 1996; Tocher 2003; Tocher and Glencross 2015), and the most common fatty acid constituent of PI, ARA (Bell et al. 1997), is an important precursor of eicosanoids, which are bioactive molecules involved in immune and inflammatory responses and renal and neural functions (Sargent et al. 1993; Bell and Sargent 2003; Tocher 2003; Tocher et al. 2008; Tocher and Glencross 2015). PA is the central precursor for the synthesis of glycerophospholipids and glycerolipids, and a signaling molecule involved in diverse cellular functions (Wang et al. 2006; Tocher and Glencross 2015). PA exhibits dynamic changes in its level in response to developmental and environmental changes (Wang et al. 2006). Therefore, a strong correlation between maternal dietary intake of PI or PA and their concentration in eggs would not be expected.

Non-genetic maternal traits, such as, age, size, and condition, could also affect egg lipid composition. For example, some studies have reported that oil globule size increased with maternal weight (Carter et al. 2015; Kolodzey et al. 2021), or age (Berkeley et al. 2004). Since the oil globule is composed mostly or entirely of neutral lipids, these studies probably point to a relationship between maternal phenotypic traits and TG, WE/SE and/or neutral lipid content of the eggs. Indeed, egg WE concentration increased with maternal length and weight in common coral trout *Plectropomus leopardus* (Carter et al. 2015). Egg ST content was negatively correlated with maternal length in the same species (Carter et al. 2015). Bransden et al. (2007) reported a decrease in TG and an increase in polar lipids in striped trumpeter eggs over the spawning season. Female traits (genetics, size, and age) may have contributed to some of the variability in our data. However, the contrast between TG concentrations of eggs from the shrimp and mackerel diet groups (from the same broodstock and in the same year) and the distinctive egg fatty acid compositions among diet groups suggest a non-genetic, dietary effect.

Significance and implications

Embryonic and early larval stages of marine fishes are characterized by high mortality rates (Houde 1997; Fuiman et al. 2015b). Optimal maternal nutritional provisioning may promote growth and survival during this critical window of development (Izquierdo et al. 2001). Characterization of egg lipid composition may provide insight into larval health, performance and likelihood of survival (Salze et al. 2005). The relationships between egg lipid class composition and egg and larval survival and performance are poorly understood and more studies are needed. Egg polar lipid content was negatively associated with larval survival in Japanese eel *Anguilla japonica* (Furuita et al. 2006). Egg TG content was negatively associated with egg viability and larval survival in European

seabass (Navas et al. 1997). Contradicting to this observation, our previous study of red drum found that larvae from eggs produced by adults on a diet of mackerel had a larger oil globule at the onset of first feeding and survived longer under starvation than the larvae from a diet of liver & squid (Hou and Fuiman 2021), suggesting a beneficial effect of themackerel diet. In the present study, we found that the mackerel diet produced eggs which contained elevated TG content. Together, these findings for red drum suggest that a higher egg TG content improves larval resistance to starvation and increases the time a larva can spend foraging during the transition to exogenous feeding and, therefore, improves survival.

It has been suggested that PUFA-rich marine pelagic eggs play an important role in the marine food web by mediating counter-gradient trophic flow from adult fish (higher consumers) to egg consumers (planktivores) (Fuiman et al. 2015b), and that the fatty acid profiles of those eggs can be useful for understanding trophic linkages and food web dynamics (Fuiman 2018; Hou et al. 2020). It has been proposed that separating neutral and polar fractions of samples could enhance detection of dietary signals of fatty acids in consumer tissues, particularly for samples rich in polar lipids, and such a practice could lead to more robust inferences about trophic interactions based on fatty acid analysis (Couturier et al. 2020). In the present study, our comparison of fatty acid profiles of three lipid fractions (WE/SE, TG, PC) of eggs after diet shifts showed that the fatty acid profiles of egg total lipids as well as those of all three lipid classes can change markedly within 5 days of a diet change. Even though such changes were more pronounced in the neutral lipids than in the polar lipids, red drum eggs contain a high proportion of neutral lipid so that additional separation of the total lipids may not be necessary. Nevertheless, it is still possible that diet variations in the wild are more subtle than the diet manipulations in our experiments and that the fatty acid profiles of neutral lipids (or in the oil globule) would

help capture the more subtle differences in the egg profiles induced by natural changes in maternal diet.

Fatty acids have the useful property of remaining intact through the digestion process, which gives rise to the marked influence of dietary fatty acids on an organism's metabolism and structure by altering the composition of cell membranes (Tocher and Glencross 2015). During reproduction, such effects can be passed on to offspring via alteration of egg compositions. Our study provides a comprehensive assessment of how dietary lipid and fatty acids are incorporated into eggs. While the tight maternal control over egg lipid class compositions may ensure proper development of embryos and larvae, the diet-induced variations in egg fatty acid compositions may affect the larval energy budget and tissue composition, which may have consequences for survival and performance.

Table 3.1: Summary of broodstock tanks and diets.

Broodstock Diet	Broodstock Tank	No. fish	No. female	Spawn dates	Days on diet	No. spawns sampled
Shrimp	MT1	4	2	1/21/18-2/14/18	>1 yr	8
Mackerel	MT1	4	2	7/5/18-8/26/18	15-67	5
Squid	MT7	4	2	4/29/18-6/8/18	5-45	6
Liver & squid	MT7	4	2	8/9/18-9/9/18	59-90	9
Herring	MT7	4	2	5/11/20-5/27/20	12-28	6
Sardine	MT1*	4	2	4/8/20-4/13/20	15-20	3
	H4	3	1	4/5/20-4/30/20	41-66	4

*One female fish in MT1 died after completion of 2018 studies and was replaced in 2019.

Table 3.2: Lipid class concentrations (mean ± 1 S.D., mg g^{-1} dw) in different diet items.

Total lipids (TL) shown here represent the sum of all identified and quantified lipids shown, excluding unidentified components in the crude lipid extracts. n.d.: not detected.

Lipid class	Diet item					
	Mackerel	Sardine	Herring	Squid	Liver	Shrimp
WE/SE	n.d.	0.7 ± 0.2	0.7 ± 0.2	7.4 ± 2.2	1.6 ± 0.3	n.d.
TG	140.8 ± 75.7	62.6 ± 26.2	55.0 ± 36.2	22.0 ± 17.8	9.7 ± 8.7	1.0 ± 0.7
ST	4.9 ± 1.0	3.1 ± 0.9	2.8 ± 0.4	11.3 ± 1.5	8.3 ± 1.3	4.8 ± 1.4
FFA	9.4 ± 2.4	3.1 ± 0.8	2.0 ± 0.4	37.1 ± 11.5	13.9 ± 8.5	3.4 ± 0.8
PE	0.5 ± 0.3	1.7 ± 0.3	5.2 ± 1.2	8.5 ± 1.9	30.5 ± 3.8	10.3 ± 1.2
PG	n.d.	0.1 ± 0.1	0.1 ± 0.0	3.6 ± 0.8	n.d.	n.d.
PI/PA	0.1 ± 0.0	1.2 ± 0.4	1.8 ± 0.4	1.3 ± 0.3	5.1 ± 1.0	0.9 ± 0.2
PS	0.4 ± 0.1	0.9 ± 0.4	1.9 ± 0.4	2.6 ± 0.6	5.2 ± 0.8	3.0 ± 0.5
PC	4.2 ± 1.3	7.4 ± 0.4	16.8 ± 4.4	19.6 ± 2.9	58.8 ± 2.7	23.1 ± 5.4
SM	n.d.	0.6 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.5 ± 0.3	2.1 ± 0.5
LPC	0.6 ± 0.5	4.1 ± 0.8	2.7 ± 0.8	n.d.	n.d.	n.d.
NL	155.1 ± 78.2	69.5 ± 27.7	60.5 ± 36.3	77.7 ± 26.8	33.5 ± 14.3	9.1 ± 2.0
PL	5.9 ± 1.6	16.0 ± 2.3	29.4 ± 7.0	36.7 ± 5.8	101.1 ± 4.7	39.4 ± 6.8
TL	161.0 ± 77.1	85.5 ± 29.9	89.9 ± 38.9	114.4 ± 23.8	134.7 ± 12.6	48.5 ± 8.8

Table 3.3: Lipid class composition (mean ± 1 S.D., % of total lipids) of red drum eggs from different diet groups (n = 5-9 spawns).

Boldface type indicates lipid class concentrations that differ significantly among diet groups (ANOVA, FDR = 0.05). For each lipid class, values that share the same superscript letter are not significantly different (Tukey's HSD, adjusted $P < 0.05$).

Lipid class	Diet group					
	Mackerel	Sardine	Herring	Squid	Liver & squid	Shrimp
WE/SE	24.5 ± 3.4^{ab}	23.4 ± 4.1^{b}	26.1 ± 2.2^{ab}	30.0 ± 5.2^{a}	29.6 ± 2.6^{a}	25.6 ± 2.6^{ab}
TG	36.0 ± 3.7^{a}	31.5 ± 5.9^{ab}	29.5 ± 3.5^{ab}	30.2 ± 2.2^{ab}	26.2 ± 3.0^{b}	27.9 ± 5.4^{b}
ST	3.4 ± 0.2^{b}	3.8 ± 0.3^{ab}	4.1 ± 0.5^{a}	3.7 ± 0.3^{ab}	4.2 ± 0.3^{a}	3.3 ± 0.4^{b}
PE	3.5 ± 0.5	3.6 ± 0.4	4.0 ± 0.9	4.2 ± 0.9	4.1 ± 0.6	3.9 ± 0.6
PI/PA	2.6 ± 0.6^{a}	1.2 ± 0.2^{b}	1.4 ± 0.2^{b}	2.0 ± 0.4^{ab}	2.6 ± 0.9^{a}	2.6 ± 0.6^{a}
PS	0.7 ± 0.2	0.6 ± 0.1	0.9 ± 0.2	1.0 ± 0.3	0.8 ± 0.3	0.8 ± 0.2
PC	22.1 ± 2.6	22.5 ± 1.4	25.8 ± 2.3	22.5 ± 3.7	22.7 ± 2.8	20.5 ± 3.1
NL	63.9 ± 6.6	58.7 ± 9.6	59.7 ± 5.0	63.9 ± 5.1	60.1 ± 4.5	56.8 ± 6.9
PL	28.9 ± 2.5	28.0 ± 1.8	32.1 ± 3.4	29.7 ± 4.7	30.1 ± 3.0	27.8 ± 3.8

Table 3.4: Coefficients of variation (CV) for groups of fatty acids in eggs by lipid class.

Lipid class	Coefficients of variation					
	SFA	MUFA	PUFA	n-3 HUFA	n-6 HUFA	HUFA
Total lipid	5.3%	19.6%	14.8%	24.7%	60.2%	17.3%
PC	5.3%	17.3%	5.6%	19.1%	61.6%	7.6%
TG	10.9%	20.4%	20.9%	31.7%	63.4%	25.9%
WE/SE	27.7%	18.8%	23.1%	35.0%	71.9%	27.0%

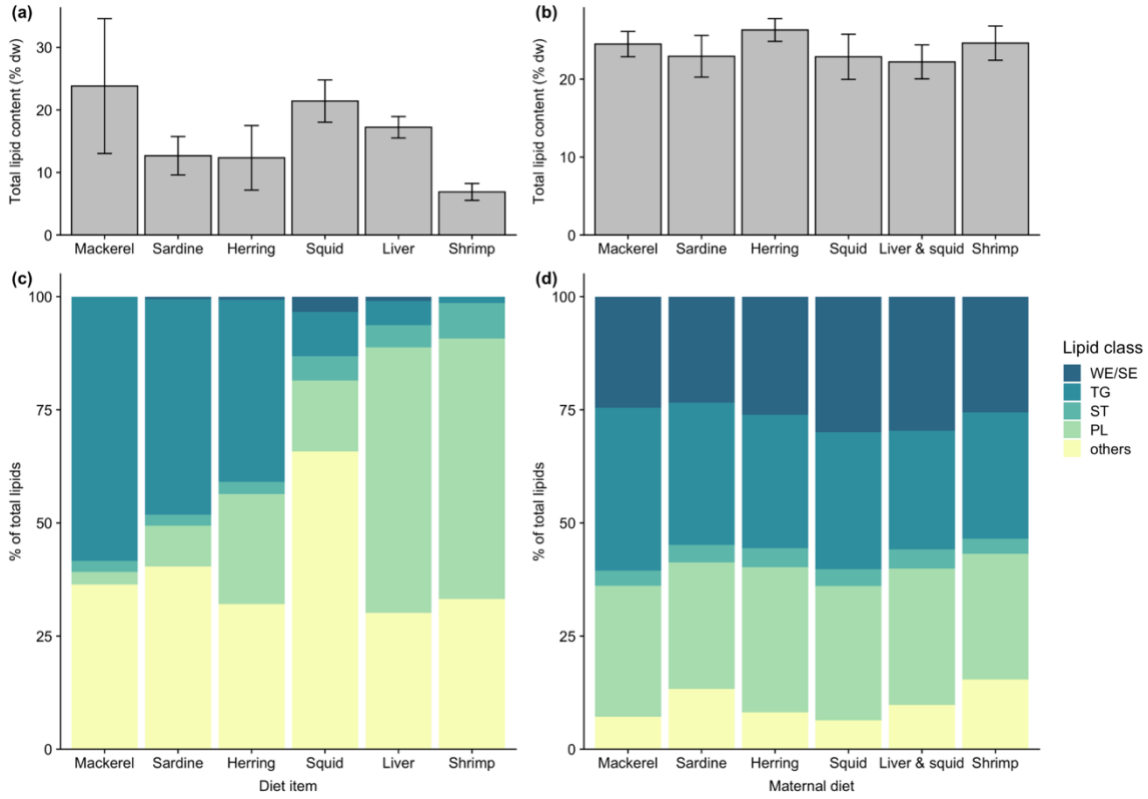

Figure 3.1: Lipid content (determined gravimetrically; % dry weight) of (a) diet and (b) eggs; and lipid profiles (% total lipids) of (c) diet and (d) eggs.

From top to bottom, the bars in (c) and (d) represent: WE/SE, TG, ST, PL (sum of PE, PI/PA, PS, PC), and 'others' (unquantifiable and unidentified lipid classes in the crude lipid extracts). For (c), 'others' include lipid classes that were not present or quantifiable in eggs, including FFA, LPC, PG, SM. For full lipid class profiles, see Tables 3.2 and 3.3.

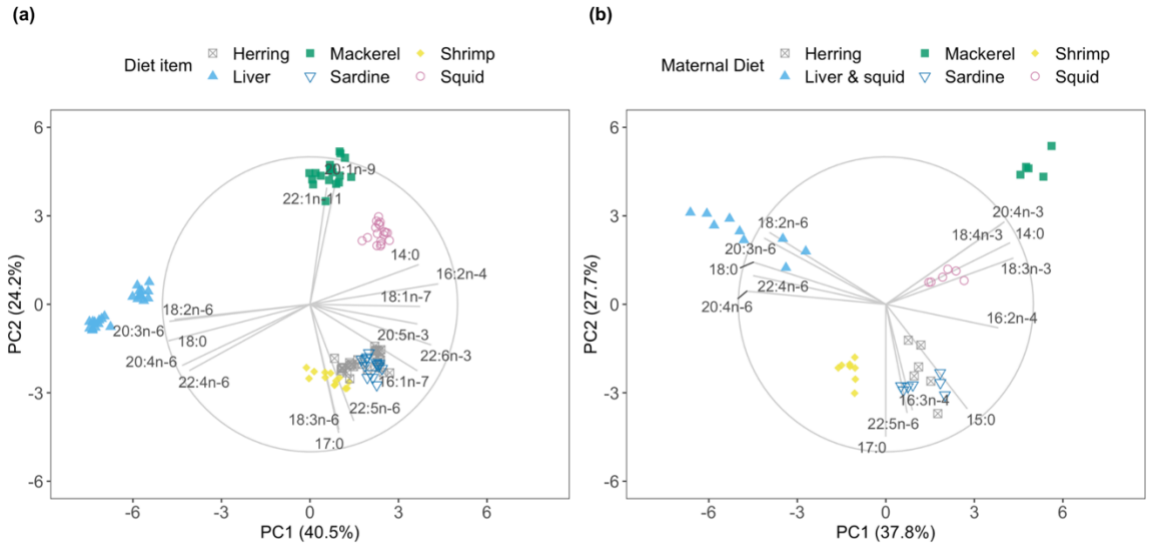

Figure 3.2: Principal components analysis of fatty acid concentrations (% total fatty acids) in total lipids of (a) diet items and (b) eggs.

Principal component loadings for the most influential fatty acids (|loadings| > 0.7) are shown by gray lines, with a circle of radius 1 for reference. All fatty acid loadings are in Tables B5 and B8. Colors and symbols indicate diet items or egg diet groups (grey square cross: herring; blue filled triangle: liver (or liver & squid); green filled square: mackerel; dark blue open triangle: sardine; yellow filled diamond: shrimp; pink open circle: squid).

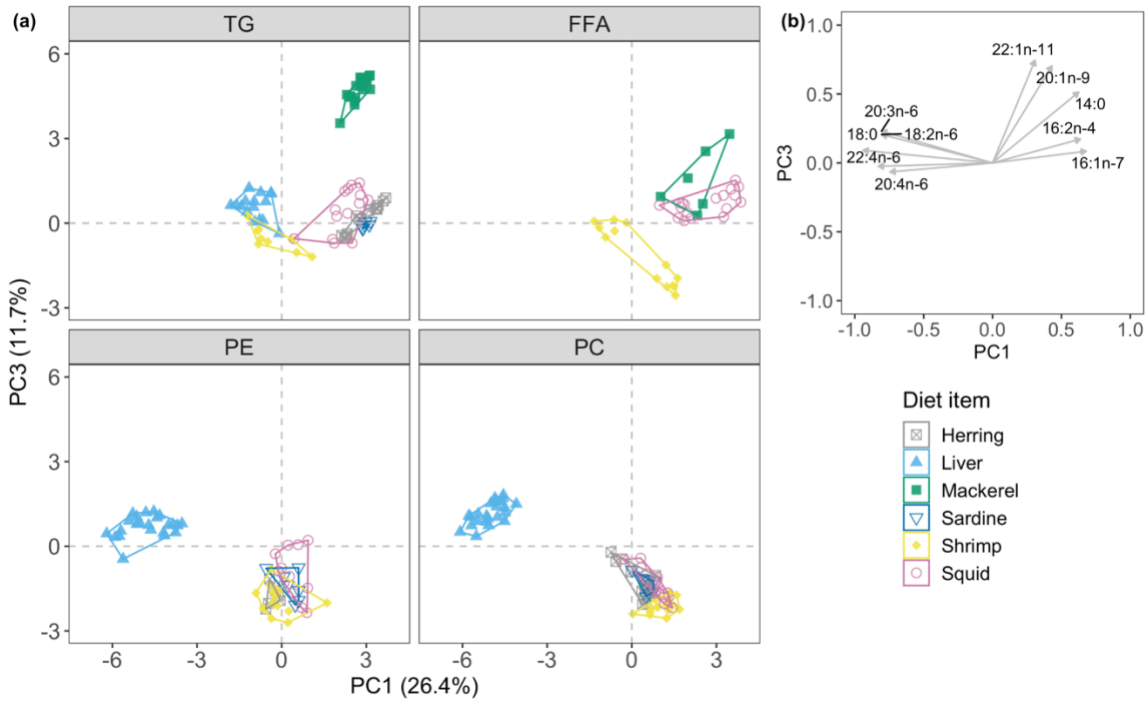

Figure 3.3: (a) Principal components analysis of diet fatty acid concentrations (% total fatty acids) in major lipid fractions. (b) Principal component loadings for the most influential fatty acids (|loadings| > 0.6) are shown by gray arrows.

All fatty acid loadings are in Table B6. Colors and symbols indicate diet items (grey square cross: herring; blue filled triangle: liver; green filled square: mackerel; dark blue open triangle: sardine; yellow filled diamond: shrimp; pink open circle: squid).

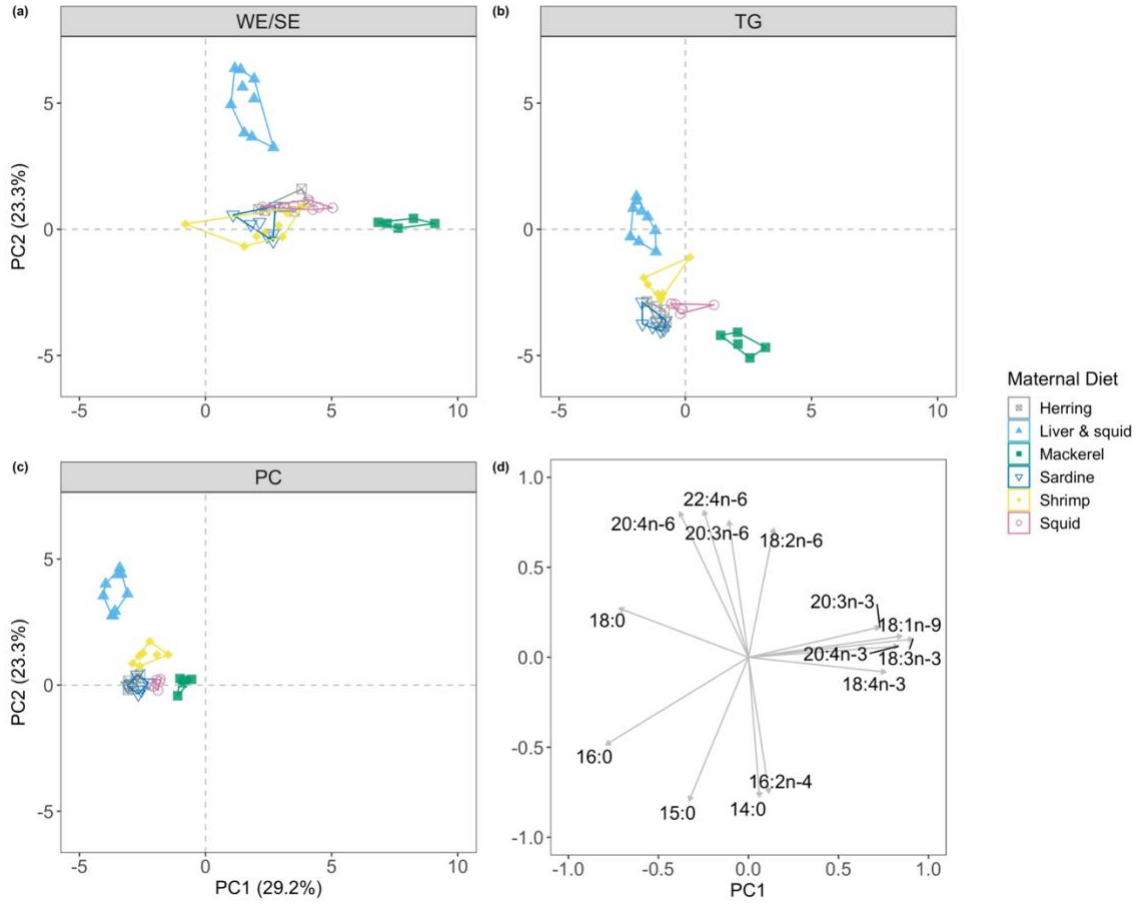

Figure 3.4: Principal components analysis of egg fatty acid concentrations (% total fatty acids) in major lipid fractions (a) WE/SE, (b) TG, (c) PC, showing differences between eggs from different diet groups.

Principal component loadings for the most influential fatty acids (|loadings| > 0.7) are shown in (d) by gray arrows. All fatty acid loadings are in Table B9. Colors and symbols indicate egg diet groups (grey square cross: herring; blue filled triangle: liver & squid; green filled square: mackerel; dark blue open triangle: sardine; yellow filled diamond: shrimp; pink open circle: squid).

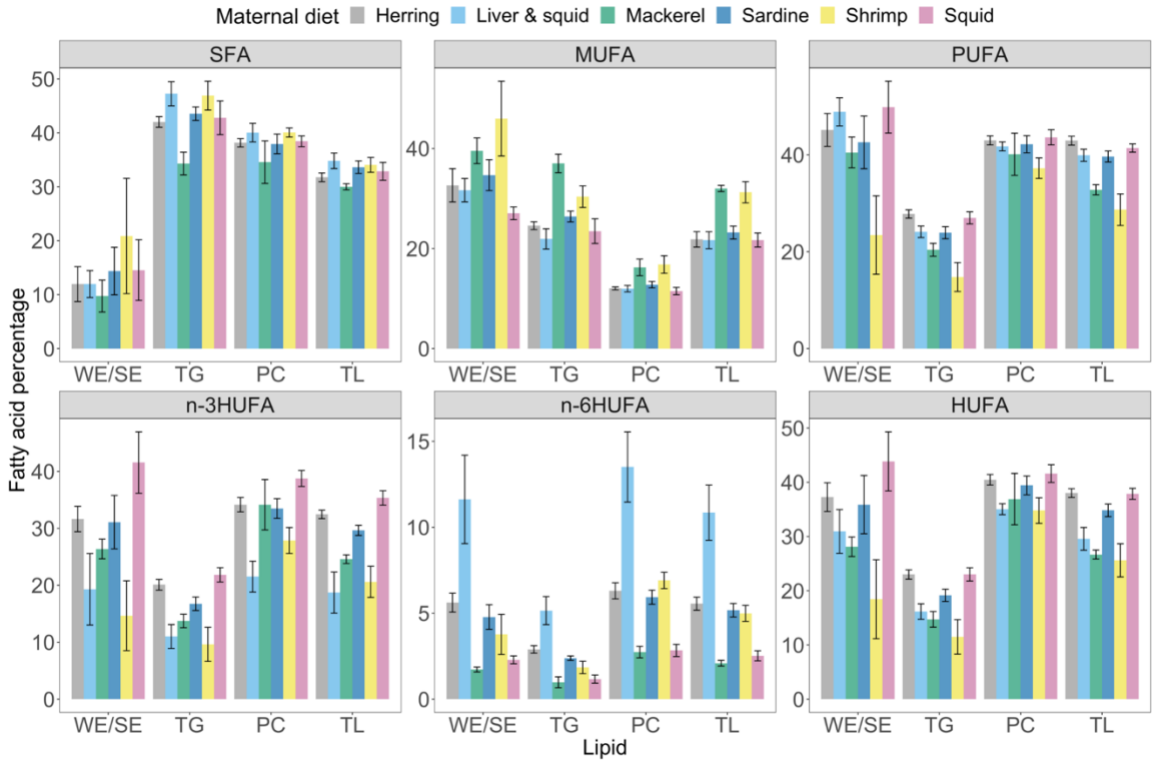

Figure 3.5: Percentages (mean ± 1 S.D.) of SFA, MUFA, PUFA, n-3 HUFA, n-6 HUFA, HUFA in WE/SE, TG, PC and total lipids (TL) of eggs from different maternal diet groups.

From left to right of each set of bars represents eggs from diets: herring, liver & squid, mackerel, sardine, shrimp, squid.

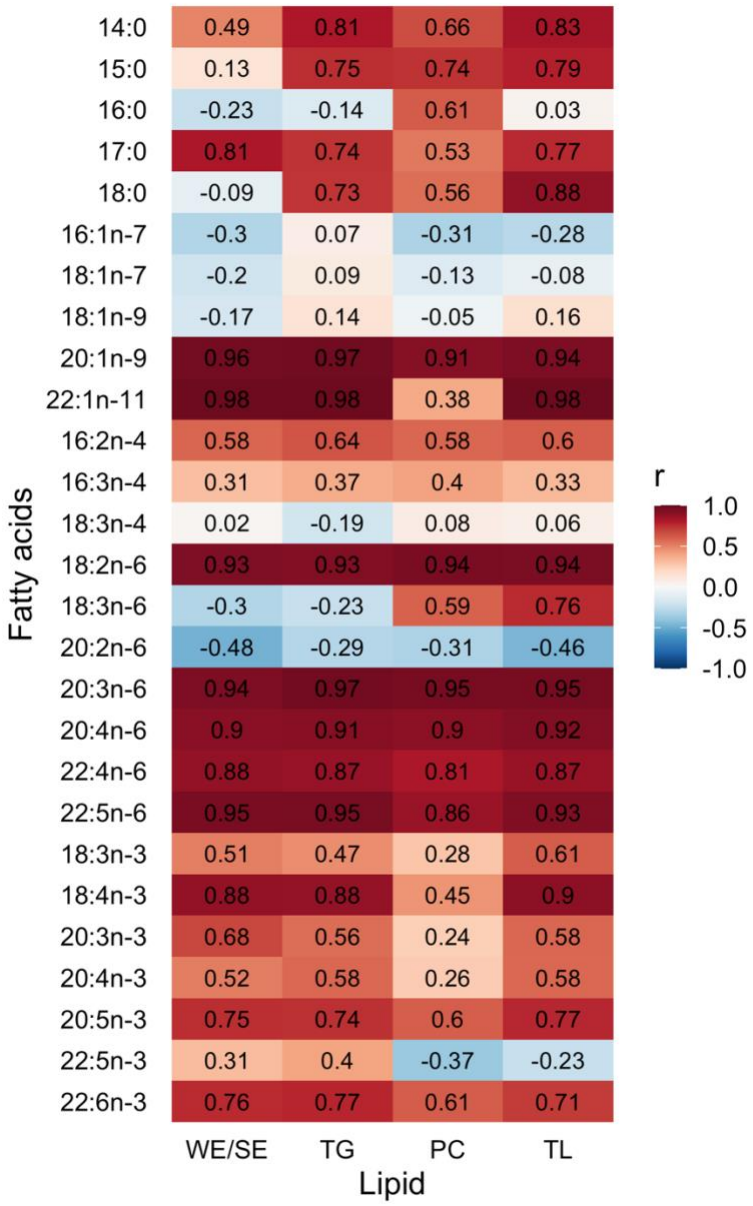

Figure 3.6: Heat map showing correlations betweens egg fatty acid concentrations (% total fatty acids) in WE/SE, TG, PC, and total lipids and mean weekly dietary intake (mg wk^{-1} $fish^{-1}$).

Color and value in each cell indicate the Pearson correlation coefficient (*r*).

Chapter 4: Maternal Diet Affects Utilization of Endogenous Lipids by Red Drum *Sciaenops ocellatus* Embryos and Early Larvae[5]

INTRODUCTION

The endogenous feeding stage of marine fishes is a period of rapid differentiation during which the structure and function of many organ systems begin to develop. During this stage, maternally derived nutritional resources (yolk and oil) are the only sources of energy and materials for metabolism, development, and growth. Recent studies have demonstrated that the composition of these endogenous nutritional resources depends, in part, on parental diet (Zakeri et al. 2014; Johnson et al. 2017; Hou et al. 2020), but whether and how such variations affect fish during this period of intense cell proliferation and differentiation is unknown.

Lipid is a major component of yolk and oil and a source of energy and structural components of cell membranes for developing embryos and larvae (Heming and Buddington 1988). The lipid content of teleost eggs varies substantially among species, ranging from 6 to 56% of dry weight (Silversand 1996). Species that produce eggs that contain one or more oil globules utilize lipids as the main metabolic fuel during embryonic and early larval development (e.g., striped bass *Morone saxatilis* (Eldridge et al. 1983), gilthead seabream *Sparus aurata* (Rønnestad et al. 1994), turbot *Scophthalmus maximus* (Finn et al. 1996), Senegalese sole *Solea senegalensis* (Mourente and Vázquez 1996), northern pike *Esox lucius* (Desvilettes et al. 1997), European seabass *Dicentrarchus labrax* (Rønnestad et al. 1998), common dentex *Dentex dentex* (Mourente et al. 1999), and white seabream *Diplodus sargus* (Cejas et al. 2004)). Their eggs are rich in lipids, > 15% of dry

[5] This chapter has been published: Hou Z, & Fuiman LA. 2021. *Comp. Biochem. Physiol. B*, 110639. https://doi.org/10.1016/j.cbpb.2021.110639. ZH designed and performed the experiments, collected and analyzed the samples, interpreted the data, and prepared the manuscript.

weight (dw), especially neutral lipids (Jaroszewska and Dabrowski 2011). Species that lack oil globules generally have lower lipid content (< 15% dw) and the lipids are predominantly polar lipids (Jaroszewska and Dabrowski 2011). In these species, polar lipids (and amino acids for planktonic eggs) are the main energy substrates (e.g., Atlantic cod *Gadus morhua* (Fraser et al. 1988; Finn et al. 1995a, b), lemon sole *Microstomus kitt* (Rønnestad et al. 1992), and Atlantic halibut *Hippoglossus hippoglossus* (Rønnestad et al. 1995)).

Fatty acids serve as energy substrates, structural components of cell membranes, and precursors of biologically active molecules, such as eicosanoids (Bell et al. 1986; Sargent et al. 1999a). Marine fish eggs are rich in fatty acids, particularly polyunsaturated fatty acids (PUFA) (Henderson and Tocher 1987). Several highly-unsaturated fatty acids (HUFA), such as arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), are required for the proper development of embryos and larvae (Izquierdo 1996; Izquierdo et al. 2000).

It has become clear that fatty acid composition of eggs within a species varies as a result of differences in maternal dietary intake, in captivity (Izquierdo 1996; Fernández-Palacios et al. 2011; Hou et al. 2020) and in nature (Fuiman 2018). For batch-spawning marine fishes, egg fatty acid composition can vary rapidly in response to changes in maternal dietary intake, within weeks in some species (e.g., red drum *Sciaenops ocellatus* (Fuiman and Faulk 2013; Hou et al. 2020); gilthead seabream (Harel et al. 1994)). The aim of the present study was to determine whether differences in egg composition (as a result of maternal provisioning of nutrients) alter the patterns of utilization of lipids and fatty acids during the endogenous feeding period. We manipulated the diets of red drum broodstock to alter egg composition and recorded changes in the utilization patterns of endogenous lipids and fatty acids during the critical window of early development. This information may help identify the connection between egg composition and larval

metabolism, its implications for larval performance, and the importance of parental dietary variations to offspring phenotype.

METHODS AND MATERIALS

Adult diet and egg composition

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin (AUP-2016-00011 and AUP-2018-00302). Four separate tanks of adult red drum (86-96 cm length, 7.6-11.1 kg weight, age undetermined; 3 to 4 fish each) were captured in nearby waters at least 1 year prior to the experiment and were maintained at the Fisheries and Mariculture Laboratory (FAML) of the University of Texas Marine Science Institute in Port Aransas, TX. They were held in 12,000 or 16,000 L recirculating tanks at a controlled temperature (24-28°C), salinity (30-36 ppt) and photoperiod (11:13 L:D during spawning). Fish were fed 3-5 times per week and were induced to spawn naturally by manipulating temperature and photoperiod (Arnold et al. 1977).

Different egg compositions were achieved by feeding adult red drum one of four diets: (1) full diet (equal parts by wet weight of shrimp *Litopenaeus setiferus* or *Farfantepenaeus aztecus*, squid *Loligo opalescens* and Spanish sardine *Sardinella aurita*), (2) mackerel diet *Scomber scombrus,* (3) shrimp diet, and (4) liver & squid diet (equal parts of beef liver and squid). These four diets were characterized by distinct fatty acid profiles (Table C1). Spawns were collected after broodstock had been fed a diet for at least 15 days (see Table 4.1 for details of broodstock spawning and egg sampling). Prior studies (Fuiman and Faulk 2013; Hou et al. 2020) suggested that the 15-day minimum period on a diet would ensure that the egg fatty acid compositions from each diet group were distinct.

Sample collection

Volitional spawning occurred at about 2000 hours and fertilized eggs were collected at 0800 hours the following day (12 hours post-fertilization, hpf). Five spawns were sampled from each diet group. Eggs from each sampled spawn were incubated in a 150-L rearing tank at a controlled temperature (27-28 °C) and photoperiod (12:12 L:D). Embryonic developmental stages were assessed by examining morphology to ensure that the time of spawning was similar for all spawns (Holt et al. 1981). Hatching was complete or near complete (>50% hatched) at 24 hpf. Red drum larvae generally start exogenous feeding around 84 hpf at 28 °C, but were not fed during this study. The final sampling point occurred between 96 and 120 hpf.

Embryos and larvae were sampled for biochemical analyses every 12 h starting 12 hpf and ending at death by starvation (96-120 hpf). They were collected from the rearing tanks using beakers, pipettes, and a mesh screen. Larvae were sacrificed by an overdose of MS-222 (0.3 mg ml^{-1}) and images of approximately 20 embryos and larvae were taken under a microscope for measurement of oil globule diameter (d) and standard length (Images for two spawns from the shrimp diet group were lost due to a technical issue). Then, approximately 100-200 eggs and 200-1000 larvae (number of larvae sampled increased as larval dry weight decreased over time) were rinsed with distilled water and frozen at -80 °C for subsequent biochemical analysis.

Biochemical analysis

Total lipid was measured by the phosphosulphovanillin method (Barnes and Blackstock 1973). Briefly, lipids were cold extracted from lyophilized and homogenized samples with 2:1 chloroform: methanol (v/v). A calibration curve was prepared by performing 1:2 serial dilutions on a cholesterol standard (Millipore-Sigma, Burlington,

MA, USA) dissolved in 2:1 chloroform:methanol (v/v). Blank, standards, and extracted lipid samples were reacted with concentrated sulfuric acid and vanillin reagent (vanillin in 4:1 85% phosphoric acid: water v/v) and were run in duplicate. Absorbance was measured using a Spectramax 190 Microplate Reader (Molecular Devices, San Jose, CA, USA) at a wavelength of 520 nm.

Fatty acid compositions of embryos and larvae were measured by gas chromatography following established methods (Faulk and Holt 2005). Briefly, lyophilized samples were homogenized and lipids were cold extracted with a known amount of tricosanoic acid (23:0) (Supelco, Inc., Bellefonte, PA, USA) as an internal standard. Fatty acid methyl esters (FAME) were prepared by saponification in potassium hydroxide in methanol, followed by transesterification with 14% boron trifluoride in methanol. FAME were dissolved in hexane before analysis by gas chromatography (Shimadzu Scientific Instruments, Columbia, MD, USA) with a flame ionization detector (FID) and a Phenomenex ZB-WAX plus capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Phenomenex, Torrance, CA, USA) or a Supelcowax 10 column. FAME were identified by comparison with commercial standards (PUFA 1-3 and Comp 37 from Supelco, Inc, Bellefonte, PA, USA, and 22:4n-6 from Cayman Chemical, Ann Arbor, MI, USA).

Variables obtained from the biochemical analyses for each sample included total lipid (expressed as mg g^{-1} dw) and the concentrations of 26 individual fatty acids (mg g^{-1} dw and % total fatty acids). Fatty acid measurements were also converted to relative concentrations, rescaled as the percentage of the value measured in the 12-hpf sample for each spawn. Relative concentrations were calculated to test whether fatty acid utilization rate was proportional to the initial level of a fatty acid (at 12 hpf). Additional variables were calculated as the sums of groups of fatty acids (saturated fatty acids (ΣSFA),

monounsaturated fatty acids (ΣMUFA), ΣPUFA, ΣHUFA, Σn-3 PUFA, Σn-6 PUFA, Σn-3 HUFA, Σn-6 HUFA [6]), and ratios of specific fatty acids (DHA:EPA, DHA:ARA, EPA:ARA, Σn-3:Σn-6 PUFA, Σn-3:Σn-6 HUFA).

Statistical analysis

Principal components analysis (PCA) with varimax rotation based on concentrations (mg g^{-1} dw) of 26 measured fatty acids (each standardized to mean = 0 and standard deviation = 1) was used to summarize the patterns of variation in fatty acid profiles for eggs and larvae among different treatment groups. One-way analysis of variance (ANOVA), followed by Tukey's tests for pairwise comparisons, were used to test the differences among diet groups in egg (12 hpf) lipid content, egg fatty acid concentrations, oil globule size at 84 hpf (after yolk exhaustion), larval standard length at 60 hpf (maximum length) and at 84 hpf (first feeding), and PCA scores. Normality and homogeneity of variance assumptions were examined. The false discovery rate ($\alpha = 0.05$) was employed for multiple comparisons (Benjamini and Hochberg 1995).

To assess changes in oil globule size, total lipid, and individual fatty acids over time, rates of change in each variable were characterized by the slopes of linear regressions for the period of 36-84 hpf. For oil globule size, the decrease over time was most strongly linear when size of the spherical oil globule was expressed as surface area (πd^2), rather than diameter or volume (i.e., rate of oil utilization was proportional to oil globule surface area). Mixed measures ANOVA was used to test for the (1) the interaction between maternal diet and age (hpf); and (2) the main effect of diet (to test for differences in mean content among different treatment groups); with maternal diet treated as the between-

[6] PUFA include FA with ≥ 2 double bonds. HUFA include FA with ≥ 20 carbon atoms and ≥ 3 double bonds in our calculation.

subjects factor, and age (hpf) treated as the within-subjects factor, and each spawn treated as a subject. Given the equal interval between each sampling point (12 hours), age (hpf) was treated as a categorical variable. When the mixed measures ANOVA interaction was significant, Tukey's test was used to test for significant differences among slopes for different diet groups (slopes were calculated for each spawn). For fatty acid variables, the FDR (= 0.05) was employed for multiple comparisons. Normality, homogeneity of variances, homogeneity of covariances, and sphericity assumptions were examined at each level of age (hpf). Because mixed measures ANOVA requires complete datasets, the two missing data (1 data point in each of two spawns, and within the linear range) for oil globule size and total lipid datasets were filled by linear interpolation. The coefficient of determination (R^2) was used to assess the relationship between standard length at 84 hpf and egg lipid content. Statistical analyses were conducted using R 4.0.3 (R Development Core Team 2020), and the rstatix package (v 0.6.0; Kassambara 2020). All figures were produced using ggplot2 package (Wickham 2016).

RESULTS

Diet effects on egg composition

Egg total lipid content (12 hpf) did not differ significantly among diet groups (ANOVA, $F_{(3,16)} = 1.89$, $P = 0.17$) and averaged 22.4 ± 2.1% dw. PCA confirmed that each adult diet treatment produced a distinctive egg fatty acid composition (Figure 4.1). The first three principal components (PC1, PC2 and PC3) accounted for 82.4% of the total variance in the data (38.6%, 35.3%, and 8.5%, respectively). PC1 separated the mackerel diet group from the liver & squid diet group, primarily as a result of differences in ARA, 22:4n-6, 20:3n-6, 18:0, EPA, 18:2n-6, 16:2n-4, 14:0, 18:3n-3, and 20:4n-3 concentrations (Figure 4.1, Table C2). PC2 scores for eggs from the full and the shrimp diet groups

differed from the other diet groups due to their concentrations of 17:0, 16:1n-7, 22:5n-6, 16:3n-4, 18:3n-4, 16:0, and 20:1n-9 (Figure 4.1a, Table C2). PC3 separated eggs from the shrimp diet group from eggs from the full diet group due to differences in 22:5n-3 (Figure 4.1b, Table C2). The differences among diet groups were significant for 24 of the 26 individual fatty acids and 11 fatty acid sums or ratios (ANOVA, FDR = 0.05, Table 4.2).

Body length

Growth in standard length of larvae was fastest between 24 and 36 hpf and increased more slowly between 36 and 60 hpf. Standard length stabilized or decreased slightly after 60 hpf (Figure 4.2). Mean length of larvae from the shrimp diet group was less than the means of all other diet groups at almost all sampling periods. Mean larval length was significantly different between the shrimp diet group and the full diet group at 60 hpf (ANOVA, $F_{(3,13)} = 4.86$, $P = 0.018$; Tukey's HSD, adjusted $P = 0.017$), but not at 84 hpf (ANOVA, $F_{(3,14)} = 3.08$, $P = 0.06$).

Lipid utilization

Most of the yolk was absorbed between 24 and 36 hpf, and yolk was fully depleted by 60 hpf (see images in Figure 4.3). Oil globule surface area did not start to decrease until after hatching (24 hpf, Figure 4.3) and oil was completely absorbed between 96 and 108 hpf, except in one spawn from the liver & squid diet group in which the oil globule was exhausted by 84 hpf. Mean surface area of the oil globule decreased linearly with time between 36 and 84 hpf (r = -0.98, -0.98, -0.98, -0.89 for the full, liver & squid, mackerel, and shrimp diet groups, respectively). There was a significant interaction between maternal diet and hpf (mixed ANOVA, $F_{(12,56)} = 2.89$, $P = 0.004$), which indicated that the rates of utilization of the oil globule (slope of regression of oil surface area on time) differed among

diet groups, with the mackerel diet group being significantly slower than the liver & squid diet group (slopes = -0.0023 ± 0.0003, -0.0021 ± 0.0003, -0.0019 ± 0.0001, -0.0017 ± 0.0001 $mm^2\ h^{-1}$ for the liver & squid, shrimp, full, and mackerel diet groups, respectively; Tukey's HSD, adjusted P = 0.007). This difference resulted in a significantly larger oil globule in 84-hpf larvae from the mackerel diet group compared with the liver & squid diet group (ANOVA, $F_{(3,14)} = 3.77$, P = 0.036; Tukey's HSD, adjusted P = 0.025; oil globule volume = $3.9e^{-4} \pm 1.2e^{-4}$, $2.9e^{-5} \pm 5.4e^{-5}\ mm^3$ for the mackerel and liver & squid diet groups, respectively; Figure 4.3).

An apparent increase in lipid content between 12 and 24 hpf (Figure 4.4) was caused by the loss of dry weight with shedding of the chorion at hatching. Subsequent depletion of lipid was linear between 36 and 84 hpf (*r* = -0.88, -0.75, -0.93, and -0.80 for the full, liver & squid, mackerel, and shrimp diet groups, respectively). Rates of lipid utilization were not significantly different among diet groups (mixed ANOVA, interaction $F_{(12,64)} = 1.25$, P = 0.27), nor did mean lipid content (between 36-84 hpf) differ significantly among diet groups (mixed ANOVA, $F_{(3,16)} = 0.94$, P = 0.47). Mean lipid content (all diet groups combined) declined from 26.2 ± 1.4% dw at 24 hpf (just after hatching) to 13.8 ± 2.2% dw at 84 hpf. The rate of lipid utilization decreased after 84 hpf, by which time the oil globule was mostly or completely absorbed (Figure 4.4). Total lipid content of eggs (12 hpf) was positively correlated with larval standard length at 84 hpf ($R^2 = 0.40$, P = 0.006; Figure 4.5).

Fatty acid utilization

The general pattern of fatty acid utilization followed that of lipid utilization. Mean fatty acid concentrations (mg g^{-1} dw) increased between 12 and 36 hpf (related to the shedding of the chorion), and then decreased steadily through 120 hpf. PCA (with varimax

rotation) of fatty acid composition showed distinct groupings of larvae from each diet group (Figure C1). PC1 explained 42.9% of the total variance and largely accounted for temporal (developmental) changes. PC1 scores were significantly correlated with age (hpf) for each diet group (R^2 = 0.89, 0.89, 0.89, 0.70 for the full, liver & squid, mackerel, and shrimp diet groups, respectively). Every fatty acid was negatively loaded on the time axis (Table C3). The strongest loadings on PC1 were mostly saturated and monounsaturated fatty acids (e.g., 16:0, 15:0, 18:1n-7, 22:5n-3, 16:1n-7, 18:1n-9, and 20:5n-3; Table C3). PC2 explained 26.2% of the total variance and separated diet groups at the early sampling periods, with mackerel at one extreme and liver & squid at the other extreme (Figure 4.6). Strongest positive loadings on PC2 were for fatty acids ARA, 18:0, and 22:4n-6. Strongest negative loadings were for 20:1n-9, 22:1n-11, and 20:4n-3 (Table C3). Trajectories of fatty acid composition (PC2) for all diet groups converged over time, reflecting a reduction in differences in the larval fatty acid composition (Figure 4.6 & C1), but PC2 scores remained significantly different among diet groups even at 108 hpf when all endogenous nutrients had been used (ANOVA, $F_{(3,12)} = 27.77$, $P < 0.001$; Figure 4.6).

There were significant differences among diet groups in the rates of utilization of 15 individual fatty acids (mixed ANOVA interaction term, FDR = 0.05): 14:0, 15:0, 16:1n-7, 17:0, 16:2n-4, 16:3n-4, 18:0, 18:2n-6, 18:3n-3, 20:1n-9, 20:3n-6, ARA, 20:4n-3, 22:1n-11, 22:4n-6. Among those 15 fatty acids, rates of utilization for 14:0, 18:3n-3, 20:1n-9, 20:4n-3, 22:1n-11 were significantly greater for larvae from the mackerel diet group than other groups (Table 4.3; Figure 4.7a). These fatty acids were in greater concentrations in eggs from the mackerel diet group than other groups (Table 4.2). Fatty acids 18:0, 18:2n-6, 20:3n-6, ARA, 22:4n-6, Σn-6 PUFA and Σn-6 HUFA were in highest concentrations in eggs from the liver & squid diet group (Table 4.2), and their rates of utilization were significantly greater in the liver & squid diet group than other groups (Table 4.3; Figure

4.7b, c). Fatty acids 16:1n-7, 17:0, and 16:3n-4 were greatest in eggs from the shrimp diet group (significantly greater than the mackerel, and liver & squid diet groups; Table 4.2), and decreased significantly faster in larvae from the shrimp diet group than the mackerel, and liver & squid diet groups (Table 4.3; Figure 4.7d). Three fatty acids and one fatty acid sum (18:0, ARA, 22:4n-6 and Σn-6 HUFA) remained relatively constant or decreased only slightly over time in most diet groups, but decreased significantly faster in the liver & squid diet group (Table 4.3; Figure 4.7c).

To test whether fatty acid utilization rate was proportional to the initial level of a fatty acid (at 12 hpf), individual fatty acid concentrations (mg g^{-1} dw) at each age were rescaled to the initial level for the same spawn (i.e., relative concentrations, expressed as % initial). No significant difference in this relative rate of utilization was found among diet groups for any fatty acids except 18:0 and 22:4n-6, which were utilized faster in the liver & squid diet group (Figure 4.8; Table C4; mixed ANOVA interaction term, FDR = 0.05). Thus, fatty acids generally were utilized in proportion to their availability.

Changes in several fatty acid ratios were examined to assess selective utilization and retention of n-3 and n-6 fatty acids. DHA:ARA decreased over time in larvae from the full and the mackerel diet groups, and remained constant in the shrimp, and the liver & squid diet groups (Figure 4.9, Table C5). DHA:EPA increased and EPA:ARA decreased over time (Table C5). The ratio of Σn-3:Σn-6 PUFA and Σn-3:Σn-6 HUFA remained constant in the shrimp, and the liver & squid diet groups but decreased over time in the mackerel and the full diet groups (Figure 4.9, Table C5).

DISCUSSION

Utilization of lipids and fatty acids

During the endogenous feeding period, most lipids and fatty acids are either catabolized for energy or incorporated into cell membranes (Heming and Buddington 1988). Lipid content decreased almost by half throughout the study, reflecting the role of lipids as an important energy source. The period of fastest growth in length coincided with the absorption of the majority of the yolk. Larvae reached their maximum length when yolk was depleted. Utilization of the oil globule began soon after hatching and ended after the point when exogenous feeding would have begun.

These results match observations of many marine species that have eggs containing oil globule(s) (Eldridge et al. 1983; Rønnestad et al. 1998; Mourente et al. 1999), In those species, yolk lipids, primarily PUFA-rich polar lipids, have important structural roles during the rapid growth in embryos and larvae (Wiegand 1996). The oil globule(s) consists primarily or completely of neutral lipids, particularly triacylglycerols and wax esters, which fuel energy demands associated with maintenance and swimming activity following yolk absorption (Kamler 2008; Jaroszewska and Dabrowski 2011). The observed reduction in standard length of red drum larvae after yolk exhaustion was probably the result of catabolism of body tissues as a result of an energy deficit caused by increased swimming activity and insufficient energy reserve (Finn et al. 1995a, 1996; Rønnestad et al. 1998), especially under starvation (Kamler 2008).

Concentrations of individual fatty acids conceivably could decrease, increase, or not change over time, depending on the extent of oxidation, conversions to other compounds (e.g., eicosanoid synthesis from C20 fatty acids), biosynthesis, and incorporation into tissues. During the endogenous feeding period of red drum,

concentrations (mg g^{-1} dw) of all fatty acids decreased. Notably, total lipid and fatty acid concentrations (expressed relative to sample dry weight) and larval dry weight undoubtedly decreased over time, indicating that the observed linear decreases in total lipid and fatty acid concentrations are the results of much faster reductions on a per-individual basis. This emphasizes that lipids and fatty acids are the predominant metabolic fuel. It is noteworthy that some fatty acids decreased more slowly than others, reflecting greater transfer to tissues (resulting in increases in their percentage composition to total fatty acids over time; e.g., DHA, ΣPUFA), whereas ΣMUFA decreased more quickly (resulting in decreases in their percentage over time) because they were a preferred substrate for energy production (see Appendix C). The preferential catabolism or retention reflects the dual roles of fatty acids (energy source and structural component).

Variations associated with yolk/oil composition

Larvae from the mackerel diet group had the largest mean oil globule after yolk exhaustion, followed by larvae from the shrimp diet group, despite a similar oil globule size in all diet groups at 12 hpf. The rank order of MUFA concentration in the eggs and at 84 hpf matched that of mean oil globule size (at 84 hpf, mean concentrations of ΣMUFA were 30.7, 27.8, 22.1, 17.0 mg g^{-1} dw in mackerel, shrimp, full, liver & squid groups, respectively). MUFA are the preferred energy substrates in red drum larvae (see Appendix C) and are commonly associated with neutral lipids, which comprise most or all of the oil globule (Eldridge et al. 1983; Silversand et al. 1996; Wiegand 1996; Mejri et al. 2018). The longer-lasting oil globule in the mackerel and shrimp diet groups may be the result of a greater amount of MUFA or neutral lipids in the oil, which may have provided more energy than the other diet groups to support development and metabolism for a longer period of time. Another possible explanation is that the embryonic nutritional environment provided

by the mackerel diet programmed a lower metabolic rate in larvae (nutritional programming; Hou and Fuiman 2020). Admittedly, a female or genetic effect cannot be ruled out, since larvae from the mackerel diet group and those from the liver & squid diet group were from different broodstock tanks (Table 4.1). However, the lack of significant difference between the mackerel group and the full diet group (same adults as the liver & squid group) suggests a non-genetic, dietary effect. In addition, the observation that larvae from the shrimp diet group had the smallest mean standard length at almost all ages suggests that the yolk in those eggs may contain less substrate for tissue synthesis,, such as essential amino acids or phospholipids (Kamler 2008) . Further investigation is needed into the effects of maternal diet on lipid class composition and energy content of eggs and its consequences for larvae (e.g., metabolic rate, size, and growth rate).

Since red drum embryos and early larvae catabolized most fatty acids in direct proportion to their availability, the differences in fatty acid compositions associated with maternal diet (represented by PC1 scores in Figure 4.1) decreased over time (represented by PC2 scores in Figure 4.6). However, despite the diminishing differences, the fatty acid profiles remained different at the time of onset of exogenous feeding and afterward. This suggests that differences in maternal nutrition changed the substrates that were available to embryos and early larvae for both energy production and building new tissues. It is known that n-3 and n-6 fatty acids compete for enzymes involved in various pathways, including eicosanoid production, fatty acid elongation and desaturation, and phospholipid synthesis (Sargent et al. 1999b). The persistent differences among diet groups in the ratios of DHA:ARA and n-3:n-6 fatty acids in larvae after yolk and oil globule depletion (Figure 4.9) probably represent differences in cellular membrane composition, which could translate into consequential changes in physiological functions, such as membrane fluidity or permeability (Harayama and Shimizu 2020), as well as ecologically relevant

performance, such as predator escape responses (Fuiman and Perez 2015; Oberg and Fuiman 2015; Burns and Fuiman 2019).

Three fatty acids – 18:0, ARA, 22:4n-6 – remained at relatively constant concentrations (mg g^{-1} dw) in larvae from the full, shrimp, and mackerel diet groups, while being catabolized in the liver & squid group during the endogenous feeding period (Figure 4.7c). Both 18:0 and ARA are known to be highly concentrated in a single phospholipid class in fish eggs – phosphatidylinositol (PI) (Bell 1989). PI generally constitutes a small proportion of egg lipids but it is conserved during development (Mourente and Vázquez 1996) because of its function as a structural component and a signaling molecule on cell membranes (Hamre et al. 2013). Moreover, conservation of ARA and 22:4n-6 may be occurring through a remodeling pathway in which n-6 PUFA deacylated from other phospholipids are selectively reacylated to PI (Tanaka et al. 2003; Leslie 2004), especially when their concentrations are low. Conservation of ARA and 18:0 during development has also been reported in Atlantic cod (Finn et al. 1995b), Senegal sole (Mourente and Vázquez 1996), and white seabream (Cejas et al. 2004). Interestingly, 18:0, ARA and 22:4n-6 were catabolized faster by larvae from the liver & squid diet group than other diet groups, even after accounting for differences in initial concentration (Figures 4.7c, 4.8c). ARA is a precursor of eicosanoids (Sargent et al. 1993), which are important for modulation of immune response, inflammatory response, renal function, and neural function (Bell and Sargent 2003; Tocher 2003). Production of eicosanoids depends upon the availability of unesterified C20 fatty acids (mainly ARA, but also EPA) (Mustafa and Srivastava 1989; Bell et al. 1995, 1996; Tocher 2003; Leslie 2004; Martins et al. 2012). Excess ARA in eggs from the liver & squid diet group might have enabled ARA to outcompete EPA during eicosanoid production, accelerating the removal of ARA from larval tissues.

Implications

Studies of fishes and other animal models have shown that maternally-derived nutrients can have effects on physiology and performance that persist into, or manifest during later life stages as consequences of nutritional programming (reviewed by Hou and Fuiman 2020). In fishes, egg fatty acid compositions have been shown to be correlated with physiological and behavioral endpoints in larvae and juveniles. For example, 21-day-old red drum larvae showed improved survival, greater accumulation of DHA and altered escape response performance associated with higher levels of several HUFA in eggs when larvae were fed a DHA-replete diet (Fuiman and Ojanguren 2011; Fuiman and Perez 2015). Similarly, juvenile Senegalese sole reared from eggs that contained higher levels of n-3 PUFA grew into significantly larger juveniles with lower incidence of caudal fin deformity than juveniles from eggs that contained lower levels of PUFA, despite the same rearing conditions (Morais et al. 2014). However, it remains unclear how differences in endogenous nutrition (caused by different maternal diets) could cause prolonged effects on an individual's performance. Previous studies have shown that early life stages are a critical window during which rapid cell differentiation and proliferation render organisms sensitive to and "programmable" by nutritional environments (reviewed by Hou and Fuiman 2020). Senegalese sole embryos and newly hatched larvae from eggs that contained lower levels of n-3 PUFA showed enhanced expression of genes involved in fatty acid biosynthesis, suggesting that nutritional programming can alter lipid metabolism as early as the embryonic and early larval period (Morais et al. 2014). The results reported in the present study, which focus on the utilization of endogenous lipids during a critical developmental window when rapid growth and differentiation occur, may provide some insight on the effects of maternal nutrition on early development and metabolism. This might help bridge

the gap between the cause (maternally-derived nutrients) and the consequences (metabolism and performance in later larvae) of nutritional programming.

First feeding is an especially important milestone for larvae because failure to find and consume suitable quantities of prey at that stage of development quickly results in death by starvation. Larger size at the first feeding stage generally promotes survival through improved foraging success/efficiency, predator evasion, swimming ability, and resistance to starvation (references in Anderson, 1988; Heming and Buddington, 1988; Miller et al., 1988; Fuiman and Magurran, 1994). We found that larval size at the first feeding stage was positively correlated with egg total lipid content, which was not a result of parental dietary differences, or a female effect. In addition, a larger oil globule at first feeding improves a larva's ability to withstand starvation by providing energy to continue searching for and catching prey. For example, larvae from the liver & squid diet group would have exhausted their oil globule by 86 hpf (based on the calculated rate of utilization), whereas the oil globule of larvae from the mackerel diet group would have lasted until 99 hpf. Indeed, larvae from the mackerel diet group (13.5-fold greater oil globule volume at 84 hpf and greater egg MUFA concentrations) survived longer under starvation than larvae from the liver & squid diet groups (Table 4.1). More studies are needed to tease apart the female and/or genetic effects from the maternal dietary effects on the utilization of oil globule.

Larval fish culture has been a bottleneck in aquaculture due to the difficulty of developing optimal larval diets that meet nutritional requirements and promote growth and survival (Sargent et al. 1997). Studying the utilization of endogenous nutrients during embryonic and larval development has been considered a useful approach to understand larval nutritional requirements (Fraser et al. 1988; Rønnestad et al. 1995; Cejas et al. 2004; Hamre et al. 2013). However, studies to date have largely overlooked intraspecific

variations in egg nutrient compositions associated with differences in adult diet and, therefore, the role of maternal diet in offspring quality. Results presented in this study indicate that future studies focusing on utilization of endogenous nutrients (lipids, fatty acids, proteins, amino acids, etc.) should take into account the variations in egg compositions that are under the influence of maternal diet. Those factors may change utilization rates and relative retention of specific nutrients and ultimately lead to broodstock diets that improve larval production and quality.

Table 4.1: Summary of broodstock diet treatments and spawning condition. Each row indicates a spawn.

			Number of			Spawning		Days on	Last
Spawn	Dietary group	Broodstock tank	fish	females	Spawn date	Salinity (ppt)	Temperature (°C)	dietary treatment	sampling point (hpf)
17-17	full diet	MT4	4	1	9/19/17	33.7	26.5	> 1 yr	120
17-18	full diet	MT4	4	1	9/21/17	33.7	26.5	> 1 yr	120
18-2	full diet	MT7	4	2	1/21/18	35.4	24.8	> 1 yr	108
18-4	full diet	MT7	4	2	1/26/18	36.0	26.0	> 1 yr	108
18-6	full diet	MT7	4	2	1/29/18	36.2	25.6	> 1 yr	120
18-92	liver & squid	MT7	4	2	8/9/18	33.0	25.3	59	108
18-93	liver & squid	MT7	4	2	8/10/18	32.6	25.3	60	108
18-94	liver & squid	MT7	4	2	8/13/18	33.4	25.0	33	96
18-96	liver & squid	MT7	4	2	8/18/18	33.3	25.5	68	96
18-97	liver & squid	MT7	4	2	8/19/18	33.3	25.5	59	108
18-84	mackerel	MT1	4	2	7/5/18	33.7	28.2	15	120
18-88	mackerel	MT1	4	2	7/20/18	34.2	26.6	30	108
18-91	mackerel	MT1	4	2	8/2/18	34.2	26.9	43	96
18-95	mackerel	MT1	4	2	8/17/18	36.1	26.7	57	108
18-100	mackerel	MT1	4	2	8/26/18	30.5	26.7	67	108
17-29	shrimp	MT4	4	1	11/29/17	34.1	26.6	60	120
18-1	shrimp	MT1	4	2	1/21/18	31.6	25.6	> 1 yr	108
18-3	shrimp	MT1	4	2	1/25/18	31.6	25.6	> 1 yr	96
18-8	shrimp	MT1	4	2	2/1/18	32.1	26.3	> 1 yr	108
20-1	shrimp	H4	3	1	1/9/20	31.7	24.6	65	120

Table 4.2: Fatty acid compositions (mg g^{-1} dw) of red drum eggs (chorion intact) at 12 hpf from different diet groups.

Values are means ± 1 S.D. of five spawns. Boldface type indicates fatty acid concentrations that differ significantly among diet groups (FDR = 0.05). For each fatty acid, values that share the same superscript letter are not significantly different (Tukey's HSD, adjusted $P < 0.05$).

	Diet group			
Fatty acid	Full	Shrimp	Mackerel	Liver & squid
Saturated fatty acids				
14:0	3.4 ± 1.3 ^{b}	2.1 ± 0.8 ^{b}	8.9 ± 0.9 ^{a}	1.9 ± 0.2 ^{b}
15:0	1.1 ± 0.1 ^{a}	1.0 ± 0.2 ^{a}	0.9 ± 0.1 ^{a}	0.5 ± 0.1 ^{b}
16:0	44.8 ± 4.7 ^{a}	42.8 ± 3.3 ^{a}	36.2 ± 1.9 ^{b}	40.9 ± 2.2 ^{ab}
17:0	1.3 ± 0.2 ^{a}	1.4 ± 0.3 ^{a}	0.6 ± 0.1 ^{b}	0.6 ± 0.1 ^{b}
18:0	7.6 ± 1.0 ^{b}	7.7 ± 1.0 ^{b}	6.9 ± 0.7 ^{b}	13.7 ± 2.4 ^{a}
ΣSFA	58.1 ± 6.5	55.1 ± 5.1	53.4 ± 3.2	57.7 ± 4.1
Monounsaturated fatty acids				
16:1n-7	16.7 ± 1.5 ^{b}	20.6 ± 2.1 ^{a}	9.0 ± 0.4 ^{c}	10.9 ± 2.0 ^{c}
18:1n-7	5.0 ± 0.5 ^{ab}	5.6 ± 0.6 ^{a}	4.3 ± 0.5 ^{b}	2.6 ± 0.5 ^{c}
18:1n-9	20.5 ± 1.5 ^{b}	23.7 ± 1.8 ^{b}	32.7 ± 2.0 ^{a}	21.9 ± 3.0 ^{b}
20:1n-9	0.7 ± 0.3 ^{b}	0.5 ± 0.3 ^{b}	6.2 ± 1.0 ^{a}	0.8 ± 0.1 ^{b}
22:1n-11	0.1 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	4.8 ± 0.9 ^{a}	0.1 ± 0.0 ^{b}
ΣMUFA	43.0 ± 3.2 ^{b}	50.4 ± 4.2 ^{a}	57.0 ± 3.6 ^{a}	36.4 ± 5.0 ^{b}
Polyunsaturated fatty acids				
n-4 fatty acids				
16:2n-4	1.8 ± 0.3 ^{a}	1.0 ± 0.8 ^{b}	2.0 ± 0.2 ^{a}	0.7 ± 0.1 ^{b}
16:3n-4	1.4 ± 0.2 ^{a}	1.7 ± 0.2 ^{a}	1.0 ± 0.1 ^{b}	0.6 ± 0.1 ^{c}
18:3n-4	0.3 ± 0.1 ^{a}	0.2 ± 0.1 ^{ab}	0.1 ± 0.1 ^{bc}	0.1 ± 0.0 ^{c}
n-6 fatty acids				

Table 4.2
(continued)

	Diet group			
Fatty acid	Full	Shrimp	Mackerel	Liver & squid
18:2n-6	2.5 ± 0.9 [b]	1.9 ± 0.2 [b]	3.2 ± 0.4 [b]	13.2 ± 3.5 [a]
18:3n-6	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
20:2n-6	0.2 ± 0.1 [c]	0.2 ± 0.1 [bc]	0.4 ± 0.1 [ab]	0.5 ± 0.1 [a]
20:3n-6	0.3 ± 0.1 [b]	0.3 ± 0.1 [b]	0.3 ± 0.0 [b]	2.9 ± 0.8 [a]
20:4n-6 (ARA)	4.2 ± 0.5 [b]	5.7 ± 0.3 [b]	2.0 ± 0.3 [c]	9.4 ± 1.8 [a]
22:4n-6	1.1 ± 0.1 [bc]	1.4 ± 0.2 [b]	0.7 ± 0.1 [c]	3.4 ± 0.7 [a]
22:5n-6	1.2 ± 0.1 [a]	1.3 ± 0.3 [a]	0.8 ± 0.1 [b]	0.9 ± 0.1 [b]
Σn-6 PUFA	9.7 ± 0.8 [b]	11.1 ± 0.7 [b]	7.4 ± 0.8 [b]	30.4 ± 6.7 [a]
Σn-6 HUFA	6.8 ± 0.6 [b]	8.8 ± 0.6 [b]	3.7 ± 0.5 [c]	16.6 ± 3.1 [a]
n-3 fatty acids				
18:3n-3	0.7 ± 0.1 [b]	0.6 ± 0.1 [b]	1.6 ± 0.3 [a]	0.6 ± 0.1 [b]
20:3n-3	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
20:4n-3	0.5 ± 0.1 [b]	0.5 ± 0.1 [b]	2.2 ± 0.2 [a]	0.5 ± 0.1 [b]
20:5n-3 (EPA)	7.5 ± 1.7 [ab]	6.0 ± 0.4 [bc]	8.8 ± 1.4 [a]	4.7 ± 0.7 [c]
22:5n-3	3.6 ± 0.4 [b]	4.8 ± 0.6 [a]	3.7 ± 0.3 [b]	4.4 ± 0.6 [ab]
22:6n-3 (DHA)	37.3 ± 1.9 [a]	29.1 ± 8.2 [ab]	28.9 ± 2.3 [b]	25.4 ± 3.1 [b]
Σn-3 PUFA	49.8 ± 3.1 [a]	41.3 ± 7.3 [ab]	45.5 ± 4.4 [a]	35.8 ± 3.9 [b]
Σn-3 HUFA	49.1 ± 3.0 [a]	40.7 ± 7.4 [ab]	43.9 ± 4.2 [ab]	35.2 ± 3.8 [b]
Other sums and ratios				
ΣPUFA	63.0 ± 4.0 [ab]	55.3 ± 7.6 [b]	56.0 ± 5.4 [b]	67.6 ± 5.2 [a]
ΣHUFA	55.9 ± 3.3	49.5 ± 7.1	47.6 ± 4.6	51.8 ± 3.2
DHA:EPA	5.1 ± 0.9 [a]	5.0 ± 1.6 [ab]	3.3 ± 0.2 [b]	5.4 ± 0.3 [a]
DHA:ARA	9.0 ± 1.1 [b]	5.1 ± 1.7 [c]	14.4 ± 1.8 [a]	2.8 ± 0.7 [c]
EPA:ARA	1.8 ± 0.5 [b]	1.0 ± 0.0 [c]	4.3 ± 0.4 [a]	0.5 ± 0.1 [c]
Σn-3:Σn-6 PUFA	5.1 ± 0.3 [b]	3.7 ± 0.8 [c]	6.2 ± 0.3 [a]	1.2 ± 0.3 [d]
Σn-3:Σn-6 HUFA	7.2 ± 0.6 [b]	4.7 ± 1.1 [c]	11.8 ± 0.9 [a]	2.2 ± 0.5 [d]

Table 4.3: Summary of fatty acid utilization rates (mg g^{-1} dw h^{-1}) during 36-84 hpf.

Values are means ± 1 S.D. of slopes of linear regressions performed for each spawn (n = 5) in each diet group. Boldface type indicates that utilization rates differ significantly among diet groups (mixed ANOVA interaction term, FDR = 0.05). For each fatty acid, slopes that share the same superscript letter are not significantly different (Tukey's HSD, adjusted $P < 0.05$).

Fatty acid	Diet group			
	Full	Shrimp	Mackerel	Liver & squid
Saturated fatty acids				
14:0	-0.06 ± 0.01 ^{b}	-0.04 ± 0.02 ^{b}	-0.14 ± 0.03 ^{a}	-0.03 ± 0.01 ^{b}
15:0	-0.02 ± 0.00 ^{a}	-0.02 ± 0.01 ^{a}	-0.01 ± 0.00 ^{ab}	-0.01 ± 0.00 ^{b}
16:0	-0.48 ± 0.11	-0.51 ± 0.22	-0.32 ± 0.07	-0.44 ± 0.08
17:0	-0.013 ± 0.003 ^{ab}	-0.015 ± 0.007 ^{a}	-0.005 ± 0.002 ^{cd}	-0.007 ± 0.002 ^{c}
18:0	0.02 ± 0.01 ^{b}	0.01 ± 0.04 ^{b}	0.01 ± 0.01 ^{b}	-0.06 ± 0.04 ^{a}
ΣSFA	-0.56 ± 0.12	-0.57 ± 0.28	-0.47 ± 0.09	-0.55 ± 0.10
Monounsaturated fatty acids				
16:1n-7	-0.28 ± 0.04 ^{ab}	-0.37 ± 0.13 ^{a}	-0.13 ± 0.02 ^{c}	-0.18 ± 0.05 ^{bc}
18:1n-7	-0.07 ± 0.01	-0.08 ± 0.04	-0.06 ± 0.02	-0.05 ± 0.03
18:1n-9	-0.26 ± 0.05	-0.33 ± 0.15	-0.39 ± 0.06	-0.28 ± 0.04
20:1n-9	-0.01 ± 0.00 ^{b}	-0.01 ± 0.01 ^{b}	-0.11 ± 0.03 ^{a}	-0.01 ± 0.01 ^{b}
22:1n-11	-0.001 ± 0.001 ^{b}	-0.002 ± 0.002 ^{b}	-0.086 ± 0.020 ^{a}	-0.002 ± 0.001 ^{b}
ΣMUFA	-0.61 ± 0.10	-0.80 ± 0.32	-0.79 ± 0.09	-0.53 ± 0.08
Polyunsaturated fatty acids				
n-4 fatty acids				
16:2n-4	-0.02 ± 0.00 ^{a}	-0.01 ± 0.01 ^{b}	-0.02 ± 0.00 ^{a}	-0.01 ± 0.00 ^{b}
16:3n-4	-0.019 ± 0.007 ^{ab}	-0.027 ± 0.014 ^{a}	-0.011 ± 0.002 ^{bc}	0.005 ± 0.003 ^{c}
18:3n-4	-0.002 ± 0.001	-0.004 ± 0.002	-0.004 ± 0.001	-0.002 ± 0.002
n-6 fatty acids				

Table 4.3 (continued)

Fatty acid	Diet group			
	Full	Shrimp	Mackerel	Liver & squid
18:2n-6	-0.04 ± 0.01 [b]	-0.03 ± 0.01 [b]	-0.04 ± 0.00 [b]	-0.20 ± 0.04 [a]
18:3n-6	-0.004 ± 0.001	-0.002 ± 0.001	-0.004 ± 0.002	-0.002 ± 0.002
20:2n-6	-0.003 ± 0.001	-0.004 ± 0.004	-0.004 ± 0.001	-0.006 ± 0.002
20:3n-6	-0.003 ± 0.001 [b]	-0.005 ± 0.002 [b]	-0.005 ± 0.001 [b]	-0.041 ± 0.011 [a]
20:4n-6 (ARA)	-0.013 ± 0.015 [b]	-0.021 ± 0.021 [b]	-0.002 ± 0.003 [b]	-0.069 ± 0.014 [a]
22:4n-6	0.001 ± 0.005 [b]	-0.004 ± 0.007 [b]	0.002 ± 0.006 [b]	-0.034 ± 0.009 [a]
22:5n-6	-0.008 ± 0.002	-0.004 ± 0.005	-0.004 ± 0.003	-0.003 ± 0.006
Σn-6 PUFA	-0.07 ± 0.02 [b]	-0.07 ± 0.04 [b]	-0.06 ± 0.01 [b]	-0.36 ± 0.08 [a]
Σn-6 HUFA	-0.02 ± 0.02 [b]	-0.03 ± 0.03 [b]	-0.01 ± 0.01 [b]	-0.15 ± 0.03 [a]
n-3 fatty acids				
18:3n-3	-0.01 ± 0.00 [b]	-0.01 ± 0.00 [b]	-0.02 ± 0.00 [a]	-0.01 ± 0.00 [b]
20:3n-3	-0.003 ± 0.002	-0.003 ± 0.003	-0.004+ ± 0.001	-0.003 ± 0.001
20:4n-3	-0.006 ± 0.002 [b]	-0.004 ± 0.002 [b]	-0.030 ± 0.003 [a]	-0.008 ± 0.002 [b]
20:5n-3 (EPA)	-0.10 ± 0.02	-0.08 ± 0.02	-0.10 ± 0.01	-0.07 ± 0.02
22:5n-3	-0.06 ± 0.01	-0.06 ± 0.01	-0.05 ± 0.01	-0.06 ± 0.01
22:6n-3 (DHA)	-0.32 ± 0.13	-0.16 ± 0.06	-0.18 ± 0.04	-0.20 ± 0.09
Σn-3 PUFA	-0.49 ± 0.17	-0.31 ± 0.05	-0.39 ± 0.06	-0.35 ± 0.12
Σn-3 HUFA	-0.48 ± 0.16	-0.31 ± 0.05	-0.37 ± 0.05	-0.34 ± 0.12
ΣPUFA	-0.60 ± 0.20 [ab]	-0.43 ± 0.08 [b]	-0.49 ± 0.07 [ab]	-0.72 ± 0.14 [a]
ΣHUFA	-0.51 ± 0.18	-0.34 ± 0.07	-0.38 ± 0.06	-0.49 ± 0.13

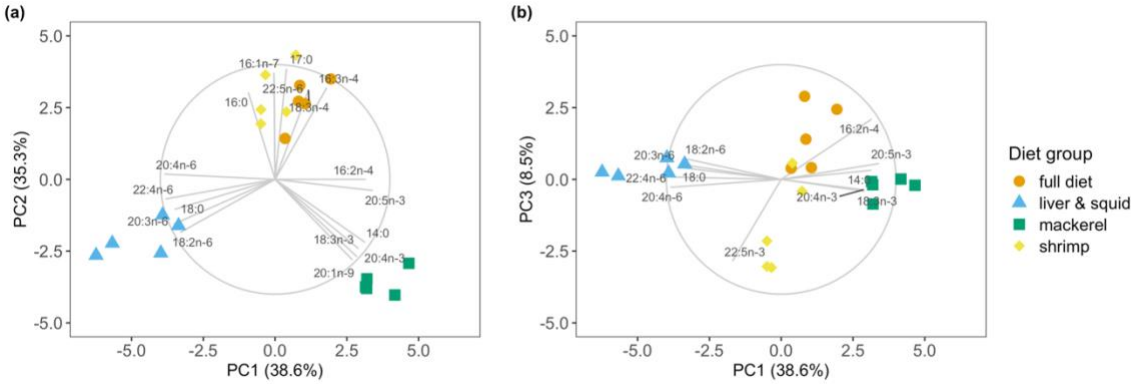

Figure 4.1: Principal components analysis of egg fatty acid concentrations (mg g^{-1} dw) showing differences in principal component scores (PC1, PC2, PC3) between adult diet groups.

Principal component loadings for the most influential fatty acids are shown by gray lines, with a circle of radius 1 for reference. All fatty acid loadings are in Table C2. Colors and symbols indicate adult diet groups (orange circle: full diet, blue triangle: liver & squid diet, green square: mackerel diet, yellow diamond: shrimp diet).

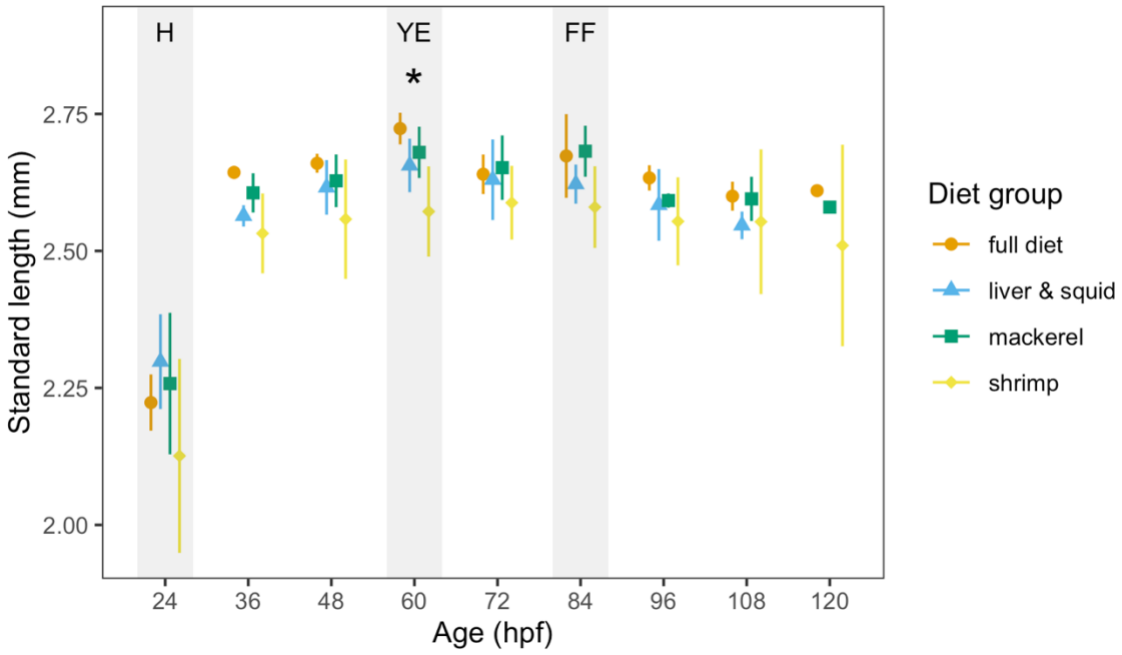

Figure 4.2: Mean standard length (± 1 S.D.) of red drum larvae (n = 5 spawns) from adults fed different diets.

An asterisk indicates that standard length differed significantly among diet groups at 60 hpf (ANOVA, $P < 0.05$). Note that the mean for each diet group were calculated for visualization, but mixed measures ANOVA reported in the text accounts for each spawn over the course of development. Shaded areas identify important developmental transitions (H, hatching; YE, yolk exhaustion; FF, first feeding).

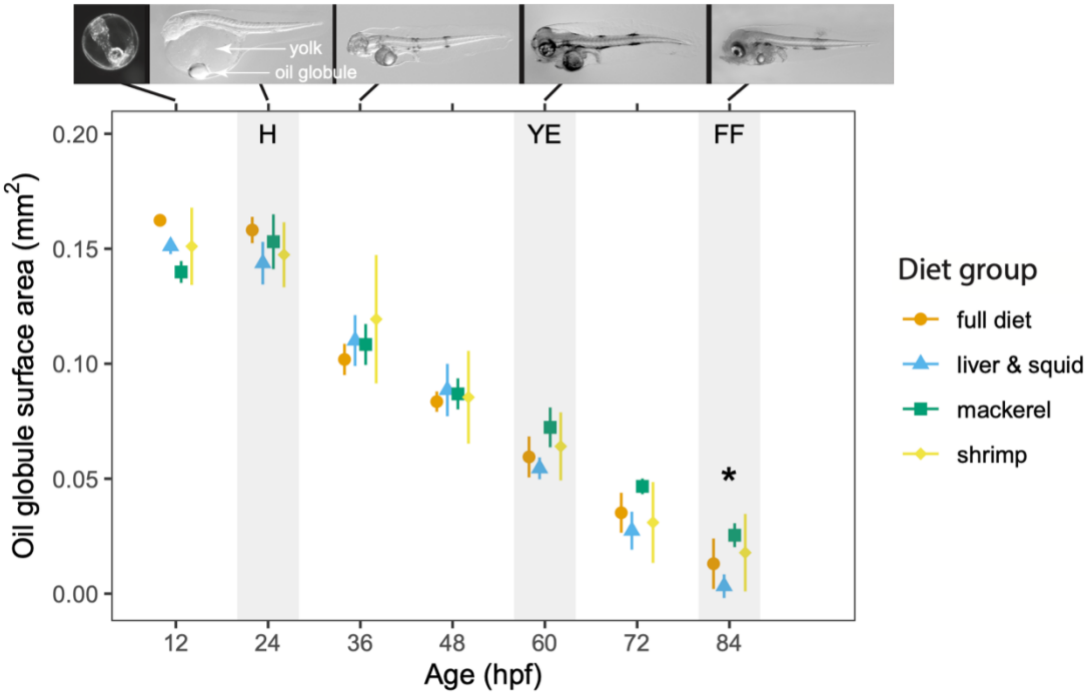

Figure 4.3: Mean oil globule surface area (mm^2; ± 1 S.D.) of red drum eggs and larvae (n = 5 spawns) from adults fed different diets.

Rates of utilization of the oil globule (between 36-84 hpf) were significantly affected by maternal diet (mixed ANOVA, interaction $P < 0.05$). An asterisk indicates oil globule surface area differed significantly among diet groups at 84 hpf (ANOVA, $P < 0.05$). Shaded areas identify important developmental transitions (H, hatching; YE, yolk exhaustion; FF, first feeding). Images of larvae at 24, 36, 60, and 84 hpf show extent of yolk and oil globule, and state of development. Photo credit: Cypress Hansen.

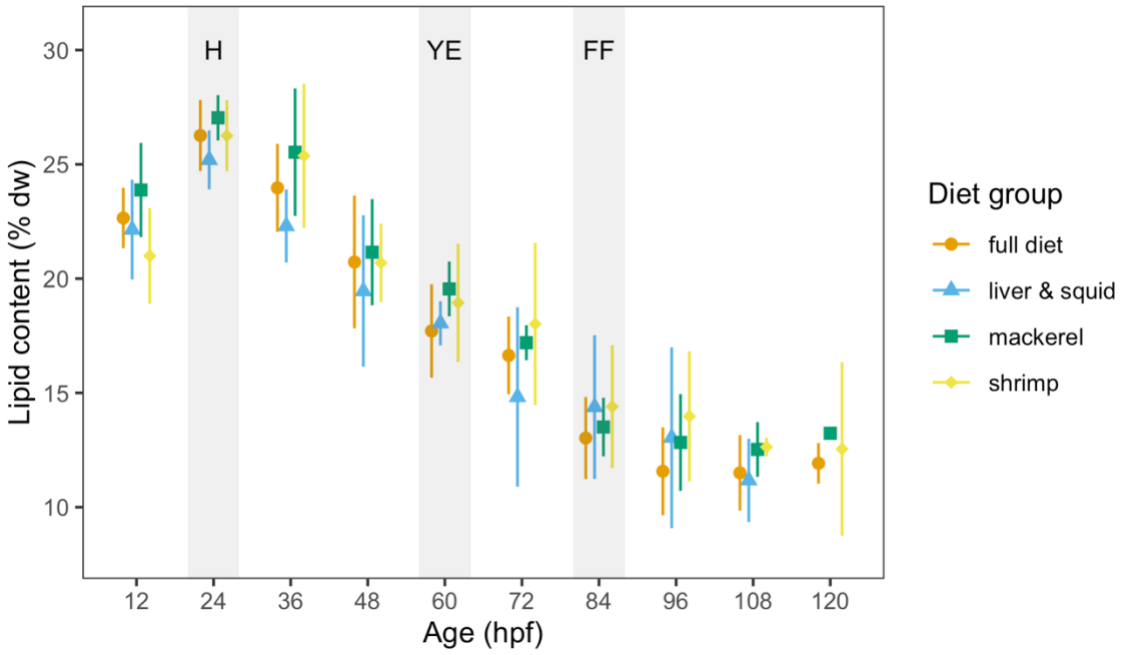

Figure 4.4: Mean lipid content (% dw; ± 1 S.D.) of red drum eggs and larvae (n = 5 spawns) from adults fed different diets.

Shaded areas identify important developmental transitions (H, hatching; YE, yolk exhaustion; FF, first feeding).

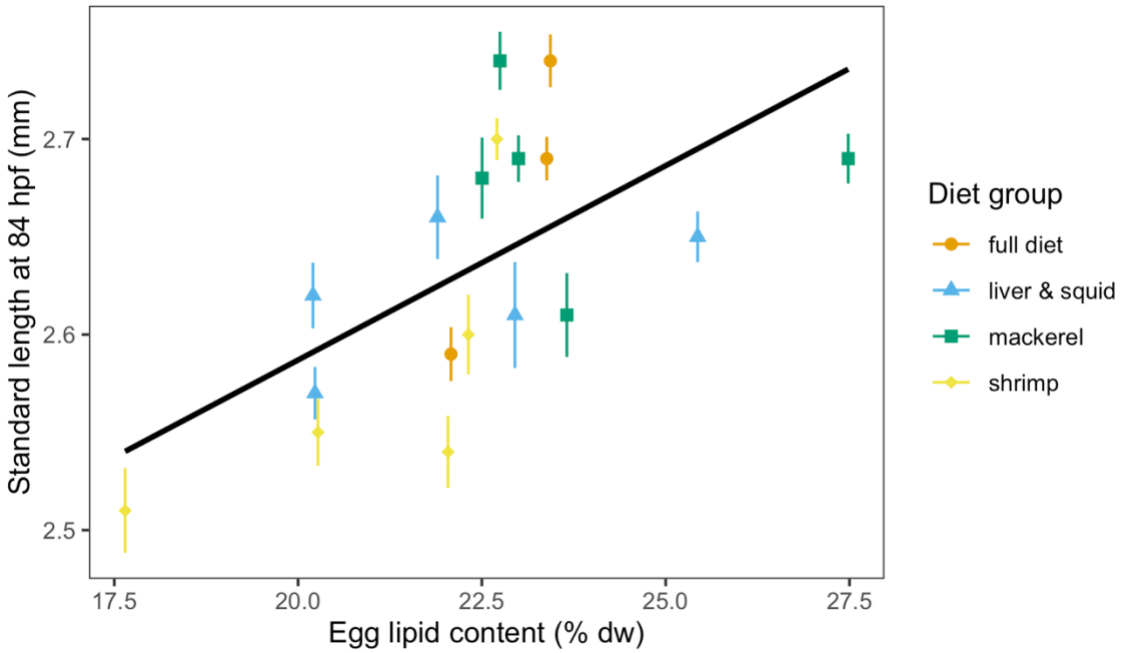

Figure 4.5: Relationship between standard length of red drum larvae at 84 hpf (± 1 S.E., n = 7-19 larvae for each spawn) and lipid content of eggs (at 12 hpf) (R^2 = 0.40, $p < 0.05$).

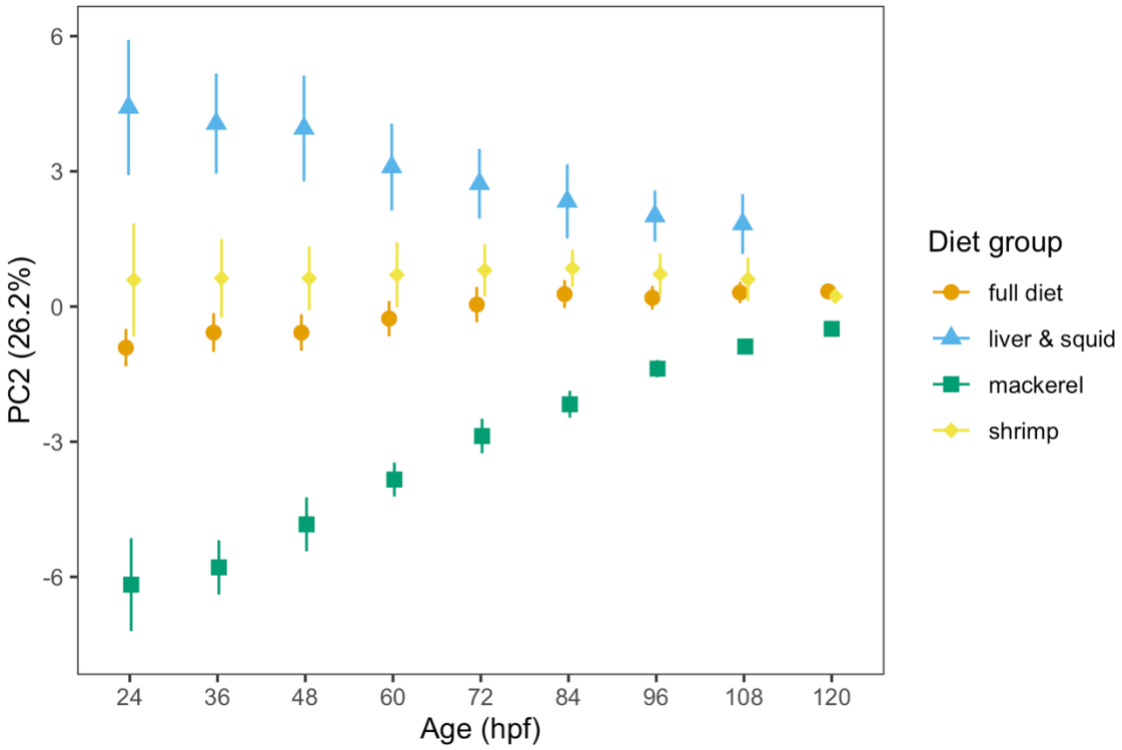

Figure 4.6: Changes in fatty acid composition (mean principal component 2 (PC2) score [± 1 S.D.]) of larvae from different diet groups over time.

Principal components analysis (with varimax rotation) based on concentrations of fatty acids (mg g^{-1} dw) in larval samples between 24-120 hpf. PC2 scores were significantly different among diet groups at the later sampling points. All fatty acid loadings are in Table C3.

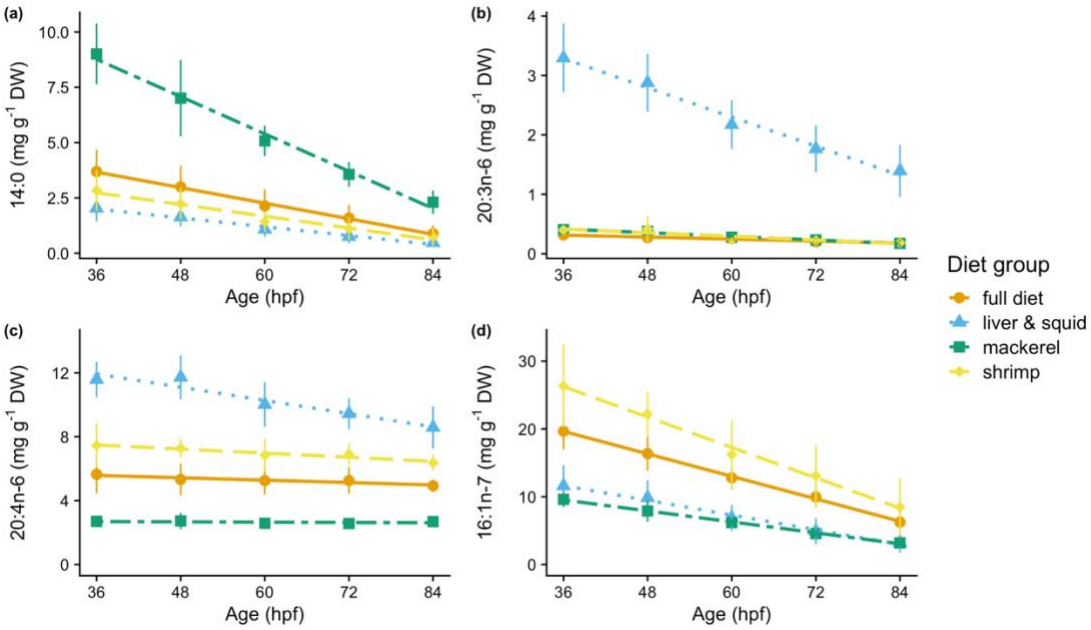

Figure 4.7: Changes in mean concentrations of selected fatty acids (mg g^{-1} dw; ± 1 S.D.) during 36-84 hpf.

Rates of utilization of fatty acids (mg g^{-1} dw h^{-1}) (a) 14:0, (b) 20:3n-6, (c) 20:4n-6, and (d) 16:1n-7 differed significantly among diet groups (FDR = 0.05). Note that the y-axis scales differ in each panel. Complete results for regression slopes and mixed ANOVA are in Table C3. Colors and line types indicate adult diet groups (orange circle and solid line: full diet; blue triangle and dotted line: liver & squid diet; green square and two-dash line: mackerel diet; yellow diamond and long-dash line: shrimp diet).

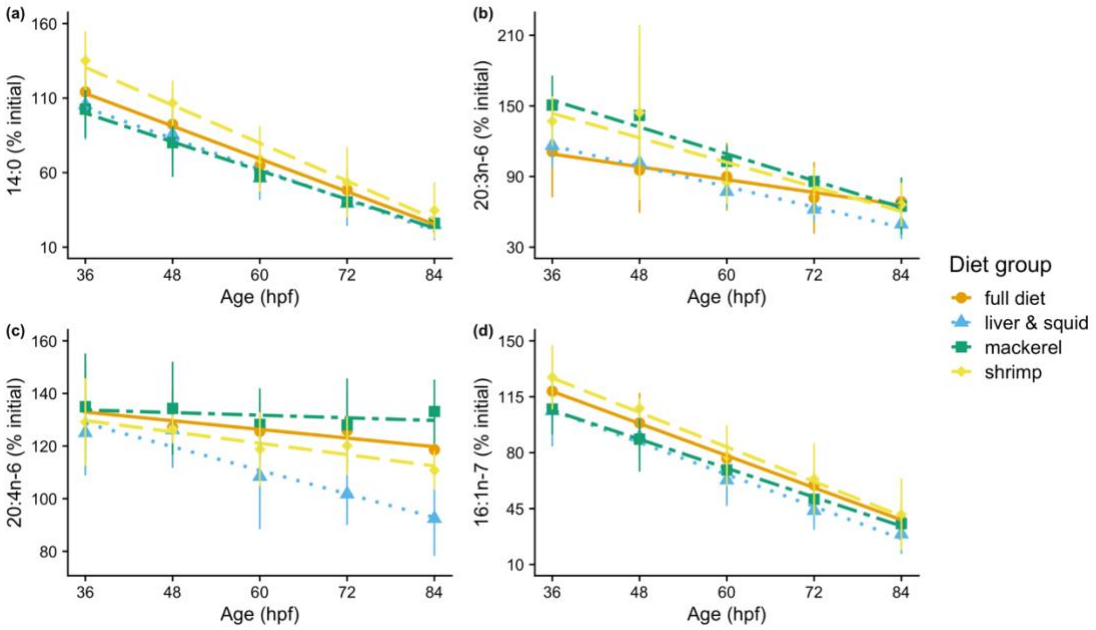

Figure 4.8: Changes in relative concentration of selected fatty acids (% initial; ± 1 S.D.) during 36-84 hpf.

Relative rates of utilization (% initial h^{-1}) of fatty acids (a) 14:0, (b) 20:3n-6, (c) 20:4n-6, and (d) 16:1n-7 did not differ significantly among diet groups (FDR = 0.05). Arachidonic acid (20:4n-6; in c), as well as 18:0 and 22:4n-6, were utilized at faster rates in the liver & squid diet group after accounting for differences in initial concentration. Relative concentrations are greater than 100% at 36 hpf because of decreased sample dry weights after shedding of the chorion at hatching. Note that the y-axis scales differ in each panel. Complete results for regression slopes and mixed ANOVA are in Table C4.

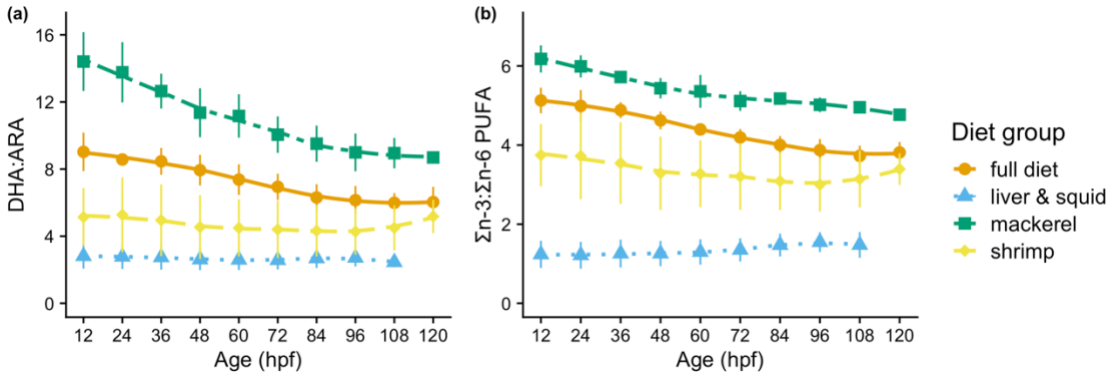

Figure 4.9: Changes in ratios of (a) DHA:ARA and (b) Σn-3:Σn-6 PUFA (± 1 S.D.) during 12-120 hpf.

Ratio and rates of change during 36-84 hpf differed significantly among diet groups (FDR = 0.05). Note that the y-axis scales differ in each panel.

Chapter 5: Nutritional programming by maternal diet alters offspring lipid metabolism

INTRODUCTION

The association between the early nutritional environment and long-term consequences for offspring is termed nutritional programming, which has been principally studied in mammalian models (Symonds et al. 2009; Ozanne 2015). In recent years, similar transgenerational effects of early nutrition, both endogenous (maternally derived) and exogenous (larval feeding) nutrients, have been reported in fishes (Panserat et al. 2019). The consequences for offspring growth, survival, neural development and nutrient metabolism have been documented (Vagner et al. 2007; Lund et al. 2012; Morais et al. 2014; Fuiman and Perez 2015; Izquierdo et al. 2015).

Lipid homeostasis is crucial for the development and functioning of many important physiological processes. Dietary lipids and fatty acids are important for larval survival, growth, stress resistance, swimming, feeding, and escape behavior (Hamre et al. 2013). Therefore, lipid nutrition in fishes has received much research interest (Sargent et al. 2002). The first-feeding diet can modify lipid metabolism in fishes and have important, long-term consequences. For example, a diet deficient in highly-unsaturated fatty acids (HUFA) offered for 5.5 weeks at first feeding upregulated HUFA biosynthesis in 5-month-old juvenile European seabass *Dicentrarchus labrax* (Vagner et al. 2007, 2009). Juvenile pikeperch *Sander lucioperca* that received a HUFA-deficient diet for 3 weeks at first feeding exhibited a smaller brain and a lower concentration of docosahexaenoic acid (DHA; 22:6n-3) in the brain (4 months later) than counterparts fed the DHA-supplemented diet (Lund et al. 2012). Those malnourished fish also experienced much higher mortality when exposed to a stress challenge (Lund et al. 2012).

Contrary to the growing number of investigations of programming via nutritional intervention at first feeding, few studies have examined programming via maternal nutrition. Structures and functions of many organ systems begin to develop during the endogenous feeding period (embryonic and early larval stages), when there is substantial developmental plasticity. This makes the maternally derived nutrients in the eggs a potential source of nutritional stimuli that could permanently program offspring metabolism. Several studies have demonstrated that egg composition can affect lipid metabolism. Differences in the levels of polyunsaturated fatty acids (PUFAs) in the maternal diet of Senegalese sole *Senegalese sole* resulted in differentially expressed genes that are involved in HUFA biosynthesis in larvae and altered growth rate in juveniles (Morais et al. 2014). Zebrafish *Danio rerio* that were fed diets deficient in one-carbon micronutrients (vitamin B and methionine) produced offspring with increased hepatic lipid content and changes in the expression of various lipid metabolism genes as adults (Skjærven et al. 2018).

Fuiman and Perez (2015) were among the first to document a relationship between embryonic availability of DHA and accumulation of DHA and performance in later larvae. They showed that tissue DHA content was about two times greater in red drum *Sciaenops ocellatus* larvae that came from eggs with high DHA concentrations compared to larvae from eggs with lower levels of DHA, after larvae had fed on a high-DHA diet for several weeks (Fuiman and Perez 2015). In addition, the differential accumulation of DHA was associated with aspects of larval performance that are critical to survival, such as growth, routine swimming, and predator escape response (Fuiman and Ojanguren 2011; Fuiman and Perez 2015; Perez and Fuiman 2015). However, while egg and larval DHA levels were implicated, respectively, as the stimulus and consequence of nutritional programming in red drum, the previous study did not examine the coordinated variations that occurred in

the lipid classes and associated fatty acids in eggs and larvae, which could provide more insight into lipid metabolism of larvae. The objective of this study was to investigate the transgenerational effects of maternal nutrition on offspring lipid metabolism by assessing the effects of differences in egg composition on larval lipid and fatty acid composition.

METHODS AND MATERIALS

We conducted two experiments. The goal of the first experiment was to reduce the list of candidates for nutritional programming stimuli by producing spawns from a variety of maternal diets. Programming of larval lipid metabolism was assessed by larval lipid composition at 21 days post-hatching (dph). After tentatively identifying the stimulus (a maternal diet consisting of shrimp only), a second experiment was conducted to verify the programming effect of the shrimp diet on larval lipid metabolism while controlling for a possible female effect.

Broodstock care and diet treatments

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin (AUP-2016-00011 and AUP-2018-00302). Broodstock were maintained at the Fisheries and Mariculture Laboratory (FAML) in Port Aransas, TX. Fish were induced to spawn naturally by photothermal regulation (Arnold et al. 1977) and were held in 12,000- or 16,000-L recirculating tanks at a controlled temperature (24-28 °C), salinity (30-38 ppt) and photoperiod (11:13 L:D during spawning). Fish were fed 3-5 times per week until satiation. Different egg lipid compositional profiles were obtained by manipulating broodstock diets, following previous feeding protocols (Fuiman and Perez 2015).

In Experiment 1, two broodstock tanks (MT1, MT7) were fed a diet of shrimp (*Litopenaeus setiferus* or *Farfantepenaeus aztecus*), Spanish sardine (*Sardinella aurita*), Atlantic mackerel (*Scomber scombrus*), squid (*Loligo opalescens*), or beef liver supplemented with squid (Table D1). A total of 28 spawns (n = 4–9 spawns per diet group) were collected and reared under common conditions (described below) until 21 dph.

Based on the results obtained from Experiment 1, we conducted Experiment 2, a paired-design diet-shift experiment. Three red drum broodstock tanks (H3, H4, MT7) were fed a diet of only shrimp, and after five spawns were collected and reared, the diet was shifted to an oily fish (Spanish sardine or thread herring *Opisthonema oglinum*[7]). One broodstock tank (H3) stopped spawning after producing two spawns on the sardine diet. Spawns were collected and larvae were reared until 21 dph using the same method as Experiment 1.

Larval rearing and sampling

For each spawn, 5 ml of floating eggs (approximately 5000 eggs) were placed into a 150-L cone-shaped tank equipped with internal biofilters and gently aerated. Two additional samples were rinsed twice in deionized water and preserved at -80 °C until biochemical analysis.

Larvae were fed enriched rotifers (*Brachionus plicatilis* L-strain) twice daily at a concentration of 5 ml^{-1} on 3 to 11 dph. Newly hatched *Artemia* sp. nauplii were added to the diet on 10 and 11 dph. Starting 12 dph, larvae were fed enriched *Artemia* sp. nauplii twice daily at a concentration of 250-400 L^{-1}. Rotifers were enriched with Algamac 3050 (0.2 g of enrichment per one million rotifers; Aqua-fauna Bio-Marine; www.aquafauna.com) for 45-60 min prior to feeding. *Artemia* sp. were enriched overnight

[7] Types of fish feeds were switched due to a supply shortage during the 2020 pandemic.

with Algamac 3050 (0.3 g of enrichment per 100,000 *Artemia* sp.). Temperature, salinity, and photoperiod were maintained at 27.5 ± 0.5°C, 31 ± 2 ppt, and 12:12 L:D, respectively. At 21 dph, at least 40 larvae were sampled, euthanized by an overdose of MS-222 (0.3 mg ml^{-1}), rinsed twice in deionized water and frozen at -80 °C until analysis. Images of approximately 20 larvae were taken under a microscope for measurement of standard length. Egg and larval samples from Experiment 1 were used for fatty acid and untargeted lipidomic analyses. Egg and larval samples from Experiment 2 were used for fatty acid and lipid class analyses.

Biochemical analysis

Fatty acid analysis

Fatty acid compositions of eggs and larvae were measured by gas chromatography following established methods (details in previous chapters; Faulk and Holt 2005). Fatty acids (in total lipids) were quantified as mg g^{-1} dw and % total fatty acids (referred as "concentration" and "composition", respectively).

Untargeted lipidomic analysis

Samples were homogenized in a Precellys®24 homogenizer equipped with Cryolys® system (Bertin Corp, Rockville, MD, USA) by adding 1 ml cold 1:1 methanol:water with 10 mM ammonium bicarbonate and operating at 6500 rpm × 2 cycles × 20 seconds for four times. Homogenates were transferred into glass vials and washed twice with 500 µl of 1:1 methanol:water with 10 mM ammonium bicarbonate. Then, 2 ml of chloroform with butylated hydroxytoluene (BHT) as an antioxidant were added to each vial. The nonpolar fractions of samples were dried using a CentriVap refrigerated vacuum concentrator (Labconco, Kansas City, MO, USA) and re-constituted in 6:3:1 acetonitrile:methanol:choloroform with a mix of deuterated complex lipids (10%

SPLASH LIPIDOMIX Mass Spec Standard, Avanti Polar Lipids, Alabaster, AL, USA). Samples were loaded in a 96-well PCR plate for injection.

Untargeted lipidomics analysis was performed on a high-resolution Hybrid Quadrupole-Orbitrap mass spectrometer (Q Exactive, Thermo Scientific, Waltham, MA, USA) equipped with an automated chip-based nanoelectrospray ionization (nESI) source (TriVersa NanoMate, Advion, Ithaca, NY). The Q Exactive mass spectrometer was operated in positive and then negative full-MS modes under the following parameters: capillary temperature, 250 °C; microscans, 1; automatic gain control (AGC) target, 2e5; mass resolution, 70,000; and mass-to-charge ratio (m/z) range, 150–2000. Nanoelectrospray conditions were 30 s acquisition time per ionization mode, 5 µl injection volume, 0.4 psi gas pressure, and 1.3 kV voltage controlled by ChipSoft software (version 8.3.3; Advion). Calibration of the mass spectrometer was achieved as described in (Lu et al. 2019). Blanks were run before and after the sampling sequence, and one quality control (QC) sample was injected every six samples. All samples were kept at 4 °C during the entire sequence. After the full-MS scan, data were analyzed to generate the inclusion list for targeted MS2 with different higher energy collisional dissociation (HCD) energies for different lipid classes and ionization modes to identify lipid class and fatty acid chains. The positive mode was used for lipid class identification: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), sphingomyelin (SM), diglyceride (DG), wax ester (WE) and cholesteryl esters (CE) at 15 eV, monoglyceride(MG) at 10 eV, triglyceride (TG) at 25 eV; and the negative mode was used for fatty acid chain identification: PC, PE at 25 eV, PS, phosphatidic acid (PA) at 15 eV, PG, PI at 35 eV, and SM at 10 eV. Tandem mass spectrometry (MS/MS) data were acquired from the samples. Raw MS1 and MS2 data from non-polar fractions were analyzed using in-house MATLAB scripts. MS1 and MS2 data

were extracted, and peak candidates were selected based on the Lipid Maps database using 5ppm mass accuracy and their diagnostic fragment ions (negative or positive) at the aforementioned collision energy. Identified lipid metabolite peak intensities were standardized by sample dry weight. The concentration of a lipid class (mg g^{-1} dw) was the sum of concentrations of lipid species from the respective lipid class, which were computed based on the internal standard peak intensity.

Lipid class analysis

Lipid class composition of the eggs and larvae was analyzed using UHPLC following previous methods (details in Chapter 3). Lipid class concentrations were quantified as mg g^{-1} dw. Fatty acid composition within major lipid classes were quantified as % total fatty acids.

Statistical analysis

Principal components analysis (PCA) with varimax rotation based on scaled (mean = 0 and standard deviation [S.D.] = 1) data was used to summarize egg and larval compositional profiles and to detect differences among diet groups. Probabilistic quotient normalization (PQN; Dieterle et al. 2006) was performed before scaling larval fatty acid concentration and lipidomic data. PQN has been used in metabolomics studies to account for variations in the overall concentrations of samples caused by dilutions. In our dataset, PQN removed the effect of concurrent changes in fatty acid concentrations (i.e., overall increase in lipid constituent concentrations in some samples) to enable comparison of relative changes in the concentration of each lipid constituent.

Permutational multivariate analysis of variance (PERMANOVA; with 9999 permutations) was used to test for significant differences in lipid class composition in eggs and larvae from different maternal diet groups. Multivariate homogeneity of group

dispersions was verified. When the PERMANOVA result was significant, analysis of variance (ANOVA), followed by Tukey's tests for multiple pairwise comparisons, was performed on each lipid class. One-way ANOVA and Tukey's tests were also used to test for differences in multivariate composition (PCA scores) and standard length among diet groups. Kruskal-Wallis tests and Welch one-way ANOVA were used when normality and homogeneity of variance assumptions were violated, respectively. Log-transformed data were used when both assumptions were violated. The non-parametric Mann-Whitney U-test (MWU) was used for pairwise comparisons following Kruskal-Wallis or Welch one-way ANOVA, and to test for differences in the concentrations of fatty acid and lipid species in larvae from the shrimp and non-shrimp diet groups (all non-shrimp diet groups were combined as a single group for comparison). False detection rate (FDR = 0.05) was employed for multiple comparisons (Benjamini and Hochberg 1995).

Given the apparent inter-broodstock tank variability in Experiment 2, two-way PERMANOVA and ANOVA were used to test for the effect of maternal diet (shrimp, fish) and female (H3, H4, MT7) on larval standard length, or egg and larval lipid class and fatty acid profiles. When the interaction term for two-way ANOVA was significant, simple main effects were analyzed (i.e., separate one-way ANOVA of one variable for each level of the second variable). Pearson correlation coefficient (r) was used to assess the relationship between the amount of lipid (mg larva^{-1}) and standard length of larvae from the same broodstock tank and maternal diet group. To test whether the rate of the lipid accumulation with standard length differed due to maternal diet (shrimp, fish) or female (H3, H4, MT7), larvae were grouped into 5 groups based on these two factors (group H3-fish was excluded because there were only two spawns). Analysis of covariance (ANCOVA) was used to compare the slopes of the regression of amount of lipid (mg larva^{-1}) on standard length among diet groups (interaction term). When the ANCOVA interaction term was significant

(i.e., slopes significantly different among treatments), Tukey's multiple comparison of regression slopes was performed.

Statistical analyses were conducted using R 4.0.3 (R Core Team 2020), Rcpm package (v 1.0.2; Derks 2020), exactRankTests package (v 0.8-32; Hothorn and Hornik 2021), and rstatix package (v 0.6.0; Kassambara 2020). All figures were produced using ggplot2 (Wickham 2016) and cowplot packages (v 1.1.0; Wilke 2020).

RESULTS

Experiment 1 – Egg lipid compositional profile

Untargeted shotgun lipidomics measured 275 lipid species in 10 lipid classes in red drum eggs, with 134 lipid fatty acid chains identified. There were significant differences in the lipid class profiles of the eggs from different diet groups (Table 5.1; PERMANOVA *pseudo-*$F_{(4, 23)}$ = 8.3; $P < 0.001$). Concentrations of all measured lipid classes differed significantly among diet groups except triglycerides (TG) and diglycerides (DG).

Different adult diets produced distinctive egg fatty acid profiles, as indicated by the significantly different PC1 and PC2 scores for the five groups (Figure 5.1a; ANOVA, $F_{(4, 23)}$ = 114.6, and $F_{(4, 23)}$ = 138.3, respectively, $P < 0.001$). Lipidomic profiles were equally distinctive and their patterns of variability among diet groups as expressed by PCA were very similar to those for fatty acid profiles (Figure 5.1a, b). PC1 and PC2 scores for five groups were significantly different (PC1: Welch ANOVA, $F_{(4, 10.9)} = 103.1$, $P < 0.001$; PC2: Kruskal-Wallis $X^2_{(4)} = 22.6$, $P < 0.001$).

Experiment 1 – Larval lipid compositional profile

Mean standard length for larvae at 21 dph was 7.9 ± 0.8, 8.4 ± 1.4, 8.8 ± 1.6, 8.8 ± 1.0, and 9.3 ± 1.7, and 10.5 ± 1.3 mm for the liver & squid, mackerel, squid, shrimp, and

sardine diet groups, respectively. The only significant difference in larval length among diet groups was between the liver & squid and the sardine groups (ANOVA, $F_{(4, 23)} = 3.9$, $P = 0.01$; Tukey, adjusted $P < 0.05$).

Untargeted shotgun lipidomics measured 304 lipid species in 11 lipid classes in larvae at 21 dph, with 120 lipid fatty acid chains identified. There were significant differences in lipid class profiles of the larvae from different diet groups (Table 5.2; PERMANOVA, *pseudo-F*$_{(4, 23)} = 5.5$; $P = 0.003$). While larval total polar lipid (PL) concentrations were similar across diet groups (except PA), concentrations of wax esters (WE) and TG were significantly different, leading to differences among diet groups in neutral lipid (NL) and total lipid (TL) concentrations in larvae (Table 5.2).

Larvae from the shrimp diet group differed from larvae from all other diet groups in both fatty acid profiles and lipidomic profiles despite receiving the same larval diet, as indicated by their significantly lower PC1 scores (Figure 5.2a, b; ANOVA, $F_{(4, 23)} = 7.9$, and $F_{(4,23)} = 6.5$, respectively, $P < 0.001$; Tukey, adjusted $P < 0.05$). Strongest loadings on PC1 based on fatty acid concentrations were for the fatty acids 18:4n-3, 18:3n-3, 14:0, 20:4n-3, 18:3n-6 (loadings < -0.7), and 18:0, 22:4n-6, 16:0, ARA, 17:0 (loadings > 0.7; Table 5.3). The lipid species with strong, negative loadings on PC1 based on lipidomic profile (loadings < -0.7) included 41 TG, 2 DG and 1 phosphatidic acid (PA) species (Figure D2).

Based on the PCA results, larvae were grouped into two groups, shrimp diet and non-shrimp diet, for further analysis. Mann-Whitney U-tests identified 13 fatty acids (shrimp > non-shrimp: 18:4n-3, 14:0, 18:3n-3, 20:3n-3, 20:4n-3; shrimp < non-shrimp: 18:1n-7, 17:0, 22:4n-6, 18:0, 22:5n-3, 18:1n-9, ARA, 16:0), and 109 lipid species (of 304) that were significantly different between the shrimp and non-shrimp diet groups (FDR = 0.05). Among the 109 lipid species, 53, 8, 4 and 3 were TG, DG, PA and sphingomyelin

(SM) species, respectively, and all of these were significantly higher in the shrimp diet group than non-shrimp (Figures 5.3 and D3). An additional 32 phosphatidylcholine (PC) species and 9 phosphatidylethanolamine (PE) species, respectively, were significantly different, with some higher in the shrimp diet group and some lower (Figures 5.3 and D3).

Experiment 2 – Egg lipid compositional profile

Results from Experiment 1 suggested the maternal diet of shrimp may have caused differences in offspring fatty acid and lipidomic (in particular, TG) profiles (Figure 5.2). Experiment 2 used a paired-design diet shift to verify the programming effect of the shrimp diet. When individual brookstock tanks were given the shrimp diet, then shifted to the fish diet, there were significant changes in the lipid class composition of the eggs (Table 5.4; 2-way PERMANOVA, *pseudo-*$F_{(1, 21)}$ = 3.8; P = 0.02). Wax esters/steryl esters (WE/SE) were significantly higher in eggs from the shrimp diet group than those from the fish group (Table 5.4; 2-way ANOVA, $F_{(1,21)}$ = 10.5, P = 0.004). There was also a significant female effect on egg lipid class composition (Table 5.4; 2-way PERMANOVA *pseudo-*$F_{(2, 21)}$ = 2.8; P = 0.02). Concentrations of all polar lipid classes and total polar lipids were significantly different among females (Table 5.4; 2-way ANOVA, P < 0.05).

PCA of egg fatty acid concentrations (with varimax rotation) showed changes in egg fatty acid profiles when the adult diet changed from shrimp to fish (Figure 5.4). Separation of eggs from the shrimp diet group from those from the fish diet group was primarily on PC1 (Figure 5.4). PC1 scores were significantly affected by both maternal diet ($F_{(1,21)}$ = 75.4, P < 0.001) and female ($F_{(2,21)}$ = 5.4, P = 0.01). There was a statistically significant interaction between maternal diet and female on PC2 scores ($F_{(2,21)}$ = 7.2, P < 0.01). In addition, fatty acid profiles of the major lipid classes (WE/SE, TG, PC) in eggs all changed significantly after the diet shift (Figure D4; see Appendix D).

Experiment 2 – Larval lipid compositional profile

Mean standard length for larvae at 21 dph did not differ among broodstock tanks or between diet groups ($P > 0.05$). Standard length for larvae from the shrimp and fish diet groups averaged 8.6 ± 0.5, 7.9 ± 2.1 mm for H3, 9.6 ± 1.6, 8.8 ± 1.2 mm for H4, and 8.8 ± 1.9, 8.8 ± 2.1 mm for MT7, respectively.

Within broodstock tanks, the amounts of individual lipid classes per larva (mg $larva^{-1}$) were strongly correlated with larval standard length (r = 0.78 to 0.998; Figure D5). The rates at which most lipid classes (sterol (ST), all polar lipids, and PL) increased with standard length did not differ among maternal diets or females (ANCOVA interaction > 0.05). However, the rates at which TG, NL, and TL increased with standard length differed among broodstock tanks (females), but not maternal diets (ANCOVA interaction < 0.05; Figure D5; see Appendix D). The slope for TG was significantly greater for tank H4 fed shrimp than for tank MT7 fed either diet (Tukey, adjusted $P < 0.05$). For NL and TL, the slopes for both diet groups from H4 were significantly greater than both diet groups from MT7 (Tukey, adjusted $P < 0.05$). Concentrations of total lipid and each lipid class did not vary significantly between larvae from the shrimp and fish diet groups at 21 dph ($P > 0.05$), but there was a significant female effect on TG, NL, TL concentrations of larvae (Table 5.5; $P < 0.05$).

Concentrations of fatty acids (mg g^{-1} dw) in total lipids of 21-dph larvae were summarized by PCA (Figure 5.5). Despite receiving the same larval diet, there were significant effects of both maternal diet and female on the fatty acid profiles in total lipids of 21-dph larvae. The maternal dietary effect on larval fatty acid profiles was dependent on females, as evidenced by the significant interaction between diet and female on PC1 scores ($F_{(2, 21)} = 27.9$, $P = 0.02$). There was a significant difference in PC1 scores for larvae from MT7 that were fed a maternal diet of shrimp or fish ($F_{(1, 21)} = 13.6$, $p < 0.05$). The

female effect on PC1 was significant when received the shrimp diet ($F_{(2, 21)} = 7.1$, $p < 0.05$). Strongest loadings on PC1 were for 18:2n-6, 20:4n-3, 20:3n-3, 18:3n-3 (loadings < -0.7), and ARA, 16:0, DHA, 22:5n-6, 15:0 (loadings > 0.7; Table 5.6). PC2 separated larvae from different broodstock tanks, as indicated by the significant female effect ($F_{(2, 21)} = 5.3$, $p < 0.05$), with significantly different PC2 scores for larvae from H4 compared to the larvae from the other two broodstock tanks (Kruskal-Wallis $X^2_{(2)} = 11.0$, $P < 0.005$; MWU, adjusted $P < 0.05$). The most influential fatty acids on PC2 were 22:5n-3, 16:2n-4, 20:1n-9, 22:4n-6, 18:4n-3 (|loadings| > 0.7; Table 5.6).

The effect of maternal diet on overall fatty acid concentrations in 21-dph larvae differed among females. For larvae from H3 and H4, the maternal diet of fish (sardine) was associated with overall higher concentrations of fatty acids compared to larvae from the shrimp diet groups; whereas for larvae from MT1 in Experiment 1 (equivalent to the conditions of Experiment 2) and MT7, the maternal diet of fish (mackerel and herring, respectively) was associated with an overall lower concentrations of fatty acids than larvae from the shrimp diet (Figure 5.6).

Larval fatty acid profiles in each of the three major lipid classes (PC, PE, TG) changed in response to the parental diet shift (Figure D6). The effect was strongest in larval TG (Figure D7), where the shrimp diet was associated with higher levels of most MUFA and PUFA, including 18:2n-6, 18:3n-6, 20:2n-6, 18:3n-3, 18:4n-3, 20:4n3, and lower levels of long-chain HUFA, including ARA, 22:4n-6, 22:5n-6, EPA, 22:5n-3, and DHA (Figure 5.7, Table D5). Similar but weaker patterns for these n-3 and n-6 PUFA were noted in PE, PC, and TL (Figure D7).

DISCUSSION

This study provided evidence that maternal nutrition can program lipid metabolism of red drum larvae. Specifically, (1) egg compositions were altered by changes in maternal diets; (2) larvae reared from those eggs exhibited differential accumulation of total fatty acids and differing fatty acid composition despite receiving the same larval diet for almost three weeks; (3) fatty acid profiles of TG were the most susceptible to modification by maternal diet and showed reduced levels of HUFA in larvae from a maternal diet of shrimp only; and (4) the effects of maternal diets on offspring varied among mothers.

Differential accumulation of fatty acids and lipids

There are several ways that nutritional programming could increase accumulation of fatty acids in larvae: increased appetite/intake, increased efficiency of digestion and/or absorption in the gut, or changes in the rate of fatty acid catabolism.

Appetite and food intake respond to gut-brain signals generated by energy balance, and are regulated by interactions of the sensory, endocrine, and nervous systems (Rønnestad et al. 2013; Bonacic et al. 2016). The role of maternal nutrition in regulating offspring appetite as a cause for lipid accumulation (obesity) has been widely studied in mammals. Early nutrition alters food intake through changes in the structure of the hypothalamus (cell number, innervation) and expression of hypothalamic appetite-regulating neuropeptides (Plagemann et al. 2000; Cripps et al. 2005). Rats fed a high-fat diet during gestation produced offspring with greater daily caloric intake and preference for fat, along with elevated mRNA and peptide levels of the orexigenic neuropeptide galanin and altered neurogenesis in the hypothalamus, which likely contributed to the observed greater body weight, body fat, and altered serum lipids (TG, FFA) (Chang et al. 2008). Maternal malnutrition (caloric restriction or low dietary protein), on the other hand,

has been reported to both induce hyperphagia (Vickers et al. 2000) and reduce expression of orexigenic peptides in offspring (Plagemann et al. 2000).

Appetite regulation is believed to be similar across vertebrates, but little is known about larval fishes (Rønnestad et al. 2013; Bonacic et al. 2016). Fish larvae generally feed constantly if prey are available, suggesting a lack of a functional appetite regulatory system (Rønnestad et al. 2013). However, a study reported changes in the expression of an anorexigenic peptide and an orexigenic peptide in rainbow trout *Oncorhynchus mykiss* after hatching as a result of different levels of methionine in maternal diets, with the former lasting until approximately 3 weeks after first feeding (Fontagné-Dicharry et al. 2017). It is also possible that nutritional intervention at first feeding alters food intake later in life. For example, a plant-based diet given at first feeding for 3 weeks induced greater food intake in rainbow trout when fed the same diet again at 7 months of age, like due to an altered flavor and feed preference (Geurden et al. 2013; Balasubramanian et al. 2016).

While evidence for a maternal dietary effect on nutrient retention (ingestion, digestion, absorption) in young fishes is scarce, several studies have found that the first-feeding diet programs nutrient retention. Gilthead seabream *Sparus aurata* larvae that were fed a soybean meal (SBM) for 2 weeks at first feeding exhibited decreased pancreatic enzyme activities. Two weeks after removal of the SBM diet, most enzyme activities returned but growth remained reduced (Perera and Yúfera 2016b), pointing to the possibility that even temporary modification in some physiological pathways can lead to permanent phenotypic changes. Juvenile zebrafish showed altered peptide absorption and fatty acid transport when fed a SBM diet for 3 days at first feeding, suggesting that the intestine may be susceptible to programming by the early diet (Perera and Yúfera 2016a). In the intestinal mucosal cells, hydrolyzed dietary lipids (e.g., free fatty acids) are mostly re-esterified and incorporated into chylomicrons or very low density lipoproteins (VLDL).

Enhanced gut clearance (absorption and transport of chylomicrons and VLDL) can increase ingestion and assimilation of dietary fatty acids (Hadas et al. 2003), whereas reduced fatty acid absorption and transport efficiency (e.g., bottleneck in chylomicrons synthesis) could cause accumulation of lipid droplets within the gut enterocytes, and reduce further absorption and assimilation (Morais et al. 2005). TG in chylomicrons and VLDL are hydrolyzed by lipoprotein lipase at peripheral tissue sites and absorbed for catabolism or storage (Sheridan 1988; Tocher 2003). Turkmen et al. (2017) found that a maternal diet of linseed oil (low HUFA) caused downregulation of hepatic lipoprotein lipase (*lpl*) expression in adult gilthead seabream offspring. The reduced lipid deposition into the liver is likely the cause of the observed reduction in liver lipid content (Turkmen et al. 2017). Furthermore, reduced lipoprotein synthesis in the liver could result in reduced transport of lipids to extrahepatic tissues and accumulation of lipid vacuoles in hepatocytes, and therefore, increased storage of lipids (Salhi et al. 1999). Therefore, it is possible that early nutrition programs offspring nutrient retention (absorption, transport) via actions on lipoprotein metabolism.

An increased metabolic rate may change energy expenditure of red drum larvae and decrease retention of dietary fatty acids. Studies have shown that rat offspring that were under-nourished during gestation exhibited upregulation of muscle mitochondrial β-oxidation (Lane et al. 2001) and decreased locomotor activity throughout life, which was exacerbated by a hypercaloric weaning diet (Vickers et al. 2003). A high-sucrose maternal diet induced a series of metabolic responses in rat offspring, including reduced mitochondrial CPTI (carnitine palmitoyltransferase I, an enzyme that facilitates fatty acid oxidation) activity, elevated levels of TG in the plasma and liver, and increased adipose tissue weight (D'Alessandro et al. 2014). Studies of gene expression reported downregulation of *cptI* expression in the liver in gilthead seabream (Turkmen et al. 2017)

and juvenile rainbow trout (Panserat et al. 2017), as a result of maternal linseed oil diet and vitamin-supplemented first-feeding diet, respectively, although corresponding phenotypic changes were absent in both studies.

The elevated retention of dietary fatty acids in larvae may have been used for TG synthesis, as fish larvae are generally thought to be unable to synthesize phospholipids *de novo* (Tocher 2003). Indeed, an increased supply of dietary fatty acids (a high-fat diet or an altered dietary regime) increased TG synthesis but not phospholipid synthesis in rodents (Iritani et al. 1976; Duarte et al. 2014). However, fish larvae may have limited capacity to increase TG synthesis, as lipids are likely mainly catabolized to meet energetic demands, or incorporated into the cellular membranes in the form of cholesterol and phospholipids, which accounted for 10% and 70% of total lipids of red drum larvae at 21 dph, respectively. The linear increases in the amount of different lipid classes with standard length suggest isometric lipid accumulation and that a major lipid depot has not yet developed in 21-dph larvae. Therefore, it is likely that larvae may accumulate more TG in lipid depots, such as liver, muscle, or adipose tissues, as they shift their energy allocation strategy from growth to storage as they grow (Norton et al. 2001; Litvin et al. 2011; Giraldo et al. 2017). If so, differences in TG synthesis induced by maternal diet would become more significant at later stages. In addition, it is likely that the previously established positive correlation between egg and larval DHA content (Fuiman and Perez 2015) was because a low egg DHA (similar to eggs from a shrimp diet) induced greater TG synthesis/accumulation, "diluting" the overall DHA (and other HUFA) pool, which are normally more concentrated in the phospholipids.

Interaction with genotype & other factors

Results from Experiment 2 revealed different responses among females in the changes in offspring lipid accumulation induced by maternal diet. Overall, studies of dietary effects on lipid metabolism and accumulation in animals have not produced consistent findings. This is partly because phenotypic measurements related to lipid metabolism, such as body fatty acid composition, are the results of a sequence of interdependent and cross-regulated molecular events and reflect the dynamics of various metabolic pathways that are involved in lipid homeostasis, including ingestion, digestion and absorption, lipoprotein transport and tissue uptake, oxidation, and acylation and remodeling (Tocher 2003; Ferramosca and Zara 2014). For transgenerational studies, it has been suggested that the discrepancies in phenotypic outcomes (e.g., obesity) of early nutrition could be accounted for by the timing of the nutritional stimulus relative to organogenesis (Cripps et al. 2005; Symonds et al. 2009), and by genetic, environmental and behavioral factors (Trujillo et al. 2006; Panserat et al. 2019).

Research on mammals and fishes suggests that phenotypic responses to nutritional stimuli can be affected by genotype. For example, even though a plant-based diet induced upregulation of n-3 HUFA biosynthesis in response to the dietary deficiency, total lipid and n-3 HUFA levels of muscle were highly heritable in families of Atlantic salmon *Salmo salar*, resulting in significantly different levels of n-3 HUFA in muscles between families of salmon that were all fed the same diet (Leaver et al. 2011). Changes in cholesterol metabolism and lipid transport in response to the same plant-based diet were also influenced by genotypes (Morais et al. 2011b). Transcriptomic analyses revealed both dietary and genetic effects on lipid metabolism genes (Morais et al. 2011a, 2012b). It has been acknowledged in clinical and epidemiological studies that phenotypic responses to particular diets are mediated by an individual's genetic background (Trujillo et al. 2006).

For example, a polymorphism in peroxisome proliferator-activated receptors γ (PPARγ) was found to be responsible for the different response of body mass index (increase or decrease) to a low dietary polyunsaturated:saturated fatty acid ratio (Luan et al. 2001).

However, how genetic variability interferes with the transgenerational nutritional effect is not well understood. Studies have identified associations between insulin resistance, dyslipidaemia (increased serum high-density lipoprotein, low-density lipoprotein) and birth size (a proxy for *in utero* nutrition) that are mediated by the same PPARγ gene polymorphism (Eriksson et al. 2002, 2003). Another hypothesis with growing evidence is that maternal nutrition modifies offspring genomes by epigenetic mechanisms (Ozanne 2015; Panserat et al. 2019), but evidence for epigenetic modifications in fishes is still scarce (Fontagné-Dicharry et al. 2017; Seiliez et al. 2017). Turkmen et al. (2019b) suggested that genetic variability interfered with the programming effect of maternal diet on offspring fatty acid metabolism. When adult gilthead seabream were given a plant-based diet, they showed varying levels of upregulation of *fads2* expression. In turn, gene expression (*cpt1*, *elovl6*) of their offspring also showed variations that were related to parental *fads2* expression levels (although the genetic variability or heritability of *fads2* was not investigated). Furthermore, these juvenile fishes also showed growth and methylation levels in the promoter regions of *fads2* that were correlated with the parental *fads2* expression level in addition to maternal diet (Turkmen et al. 2019b).

In our study, the duration of the maternal diet might have played a role in mediating the programming effect. In Experiment 1, the shrimp diet was provided to the broodstock tank for more than a year before the onset of spawning. In contrast, the females in Experiment 2 had only been on the shrimp diet for about 2 months and may have had body stores to draw upon for lipid incorporation, leading to the somewhat different egg fatty acid profiles from different tanks (Figure 5.4). The eggs from the same shrimp diet may also

have differed among females and between the two experiments in levels of some unmeasured nutrients or hormones. In addition, it is possible that the different types of oily fish (Atlantic mackerel, Spanish sardine or thread herring) used contributed to the incongruent results observed among broodstock tanks.

Different fatty acid composition

Although sometimes not statistically significant, there were consistent differences in the larval PUFA composition (expressed as % total fatty acids) as a result of changes in maternal diets. Larvae from the shrimp group generally showed elevated levels of n-3 and n-6 PUFA (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 18:3n-3, 18;4n-3, 20:3n-3, 20:4n-3) and reduced long-chain HUFA (ARA, 22:4n-6, 22:5n-6, EPA, 22:5n-3, DHA) in both experiments. This pattern was especially apparent in the TG fraction in Experiment 2. Variations in PUFA composition of larvae fed the same larval diet could arise from (1) HUFA biosynthesis, or (2) selective retention and incorporation of dietary fatty acids into tissues.

The fatty acids that were elevated in larvae from the shrimp diet group are in the initial steps of the n-3 and n-6 HUFA biosynthetic pathways ("precursor PUFA"), whereas the fatty acids that were reduced in those larvae were products of the same pathway ("product HUFA"). The opposing responses of these groups of fatty acids suggests that the n-3 and n-6 biosynthetic pathways were downregulated in larvae from the shrimp diet group (see Figure 2.5 for an overview of PUFA biosynthetic pathways). The key biosynthetic step between these precursor PUFA and product HUFA is mediated by Δ5 desaturase activity, which converts 20:4n-3 and 20:3n-6 into EPA and ARA, respectively, and is a rate-limiting step in the HUFA biosynthesis. The Δ5 desaturation step is relatively active in several freshwater and marine teleosts (references in Kabeya et al. 2015; Monroig

et al. 2018), but inactive in nibe croaker *Nibea mitsukurii*, which is in the same family as red drum (Kabeya et al. 2015). However, a separate study of red drum larvae reared on a larval diet with high levels of 18:3n-3 and low levels of DHA showed that 21-dph larvae from the shrimp maternal diet group had elevated levels of n-3 PUFA precursors (i.e., 18:3n-3, 18:4n-3; 20:3n-3; 20:4n-3) compared to larvae from the non-shrimp maternal diet group (Faulk and Fuiman, unpublished data), suggesting altered Δ5 desaturase activity in response to maternal nutrition similar to the present study. A direct measurement of Δ5 desaturase activity in future studies may help clarify the functionality of this enzyme in red drum.

Recent studies have challenged the generalization that marine fishes cannot convert 18:2n-6 and 18:3n-3 into downstream PUFA (Monroig et al. 2018). Existing knowledge, however, indicates highly variable biosynthetic functionalities among marine teleost species (Monroig et al. 2018). Despite the loss of *fads1* gene (encodes for Δ5 desaturase) in teleosts, sub- and neo-functionalization events have occurred within the *fads2* gene family in some fishes, which has enabled Fads2 enzymes to show both Δ6 and Δ5 desaturation activities to accomplish all the desaturation reactions from C18 PUFA into C20 – C22 HUFA (Monroig et al. 2018). Several studies have reported effects on the biosynthetic pathway of marine fish larvae as a result of differences in maternal nutrition. For example, when adult gilthead seabream were provided a linseed oil diet (high in 18:2n-6 and 18:3n-3), larvae showed increased *fads2* expression (Izquierdo et al. 2015; Turkmen et al. 2019a), and increased levels of ARA, EPA, DHA and reduction in their precursors in the liver (Turkmen et al. 2019a). Interestingly, Senegalese sole embryos and newly-hatched larvae showed an increased capacity for HUFA synthesis – elevated *Elovl 5* and Δ4

desaturase[8] expression, necessary for biosynthesis of DHA and 22:5n-6 from EPA and ARA, respectively (Morais et al. 2012a)) – in response to a lower egg DHA level, originating from the maternal diet (Morais et al. 2014). However, those changes were later reversed in older larvae (Morais et al. 2014). Similarly, HUFA deficiency in early nutrition (larval diet) induced enhanced expression of Δ6 desaturase in European seabass juveniles and higher DHA content in polar lipids, when challenged with a HUFA-deficient diet (Vagner et al. 2007, 2009).

Digestive enzymes differ in substrate and positional specificities and their functional development in fish larvae is nutrient sensitive (Lazo et al. 2011). If early nutrition alters the development or relative expression of those enzymes, it may result in differences in the retention of long-chain HUFA. For example, pancreatic lipase poorly hydrolyzes TG and DG that contain long-chain HUFA (the specificity of digestive enzymes decreases as the fatty acid chain length increases), while bile salt-activated lipase (BAL; the predominant lipolytic enzyme in fishes) efficiently hydrolyzes neutral lipids and phospholipids with long-chain HUFA (Small 1991; Olsen and Ringø 1997; Rønnestad et al. 2013). In addition, given that PUFA are generally esterified to the sn-2 position of TG and phospholipids, the different actions of 1,3-specific lipase (Small 1991; Sargent et al. 2002; Tocher 2003), 2-specific pancreatic enzyme phospholipase A2 (Rønnestad et al. 2013), and non-specific lipase (Tocher 2003) may cause different levels of PUFA hydrolysis and absorption. Furthermore, during TG synthesis in intestinal mucosal cells and at the storage site (after tissue uptake), it has been suggested that the re-esterified fatty acids are often different from the original ones (Small 1991), and it is possible nutritional programming enhances selection for HUFA during re-esterification. Further studies could

[8] Senegalese sole Δ4 desaturase also shows some Δ5 activity for the n-3 series (Morais et al. 2012a).

look into the enzymes involved in the de- and re-acylation processes (e.g., substrate-specific lipases and acyltransferases).

Another mechanism to explain reduced levels of HUFA in the shrimp diet group could be elevated peroxisomal β-oxidation of HUFA. Initial chain shortening of long-chain HUFA (> C20) during oxidation occurs in peroxisomes, after which further oxidation occurs in the mitochondria, or the shortened acyl-CoAs (i.e., PUFA, etc) are re-esterified to glycerolipids (Reddy and Mannaerts 1994). PPARs can stimulate peroxisome proliferation and increase the activities of several peroxisomal enzymes involved in fatty acid β-oxidation. PPARs have been implicated in mediating nutritional programming in various studies of mammals and fishes (e.g., Vagner et al. 2009; reviewed by Cripps et al. 2005 and Hou and Fuiman 2020). PPARs are nuclear receptors that mediate lipid homeostatic processes, including biosynthesis (Δ6 and Δ5 desaturases, stearoyl CoA Δ9 desaturase), fatty acid transport (fatty acid transport protein) and tissue uptake (lipoprotein lipase) in response to dietary lipids (Reddy and Mannaerts 1994; Tocher 2003; Patsouris et al. 2006). Future studies should examine the response of PPAR genes to nutritional programming, given their key role in regulation of HUFA synthesis and oxidation.

Nutritional programming stimulus

There are several constituents of the maternal diets (shrimp vs. fish) that could have stimulated programming effects. First, shrimp contain much less lipid than the other diets. Various studies have demonstrated the effects of dietary lipid level on lipid metabolic pathways in mammals and fishes, including transport, tissue uptake, *de novo* lipogenesis, and TG synthesis (Lu et al. 2013; Duarte et al. 2014; He et al. 2015). Shrimp also contain relatively low amounts of PUFA. Dietary PUFA can influence lipid metabolism by regulating transcription of critical genes involved in certain metabolic pathways (Tocher

2003; Ferramosca and Zara 2014). For example, dietary PUFA (especially n-3 HUFA) have been shown to suppress the expression of Δ6 and Δ5 desaturase via regulation by PPAR, and lipogenesis mediated via sterol regulatory element binding protein-1 (SREBP-1) transcription factor, but increase lipid oxidation and storage (Nakamura and Nara 2003; Tang et al. 2003; Ferramosca and Zara 2014; Duarte et al. 2014). Third, shrimp is characterized by a relatively low n-3:n-6 ratio. A diet with low n-3:n-6 ratio during gestation and lactation was associated with permanently reduced DHA levels in the rat brain (Li et al. 2006). Some studies also attributed the hypotriacylglycerolaemic (TG-reducing) effect of fish oil (relative to vegetable oil) to a high dietary n-3 HUFA content (Morais et al. 2011a, b). Fourth, there are comparatively higher levels of sterols in shrimp than fish. Dietary cholesterol can act as a ligand for SREBP and regulate biosynthesis of long-chain HUFA from C18 precursors (Leaver et al 2011), and can be metabolized into bile salts (via bile acids) to facilitate intestinal lipid absorption (Parish et al. 2008). Lastly, shrimp contain a higher amount of astaxanthin, an antioxidant, which could reduce peroxidative stress that is usually caused by high PUFA. In particular, astaxanthin has been shown to decrease plasma TG concentrations in mice (Yang et al. 2014). These dietary components may have synergistic effects on offspring lipid metabolic pathways via altered egg compositions or via epigenetic modifications to adult genomes that are passed on to the next generation.

Implications

Given the ever-increasing demand for food resources from aquaculture, nutritional programming may be a viable approach to improve production and food quality at a lower cost and with a lower environmental impact (Engrola et al. 2018; Panserat et al. 2019). Many studies are aiming to improve utilization of more sustainable, plant-based feeds as

replacements for fish-based diets through nutritional interventions during the larval period (Geurden et al. 2007, 2014; Gong et al. 2015; Rocha et al. 2016a, b; Perera and Yúfera 2016a, b; Zambonino-Infante et al. 2019). Several economically important fish species have demonstrated physiological adaptability to alternative feeds (Izquierdo et al. 2015; Turkmen et al. 2017, 2019a). The embryonic and larval periods are characterized by rapid morphological and physiological changes and great developmental plasticity (Pittman et al. 2013). Our study suggests an alternative strategy by altering maternal diet, which introduces nutritional stimuli at the start of the embryonic period. But more work is needed to identify the critical window for nutritional programming, and how long the programming effects created in this manner persist.

Dysfunction of lipid metabolism is associated with many diseases in humans. For example, accumulation of TG in adipose tissue is associated with obesity and diabetes (Athenstaedt and Daum 2006). Our study reveals the role of maternal nutrition in affecting offspring metabolism, specifically, lipid accumulation. This supports the relationship found in mammals between suboptimal fetal and early postnatal nutrition and the development of metabolic syndromes (Cripps et al. 2005; Symonds et al. 2009), and implicates changes in fundamental physiological pathways and functions due to altered lipid homeostasis.

The present study has also demonstrated that nutritional programming can influence larval utilization of exogenous nutrients, which leads to altered body fatty acid composition. Previous studies have found correlations between larval body fatty acid composition and performance of behaviors that are crucial to survival (e.g., predator evasion response; Fuiman and Perez 2015; Oberg and Fuiman 2015; Burns and Fuiman 2019). In addition, a field study documented a close correlation between interannual variations in egg composition and climate-driven changes in prey fields of adult red drum

(Fuiman 2018). Therefore, it is clear that maternal diets and egg composition vary in nature and it is likely that nutritional programming may occur, leading to consequences for larval survival and possibly recruitment success.

Table 5.1: Lipid class concentrations (mean ± 1 S.D. of spawns, mg lipid g^{-1} dw) in red drum eggs from different maternal diets in Experiment 1.

Boldface type indicates lipid class concentrations that differed significantly among diet groups (Kruskal-Wallis or Welch ANOVA, FDR = 0.05). For each lipid class, values that share the same superscript letter are not significantly different (Pairwise MWU, adjusted $P < 0.05$). Lipid classes: WE, wax ester; CE, cholesteryl ester; TG, triglyceride; DG, diglyceride; MG, monoglyceride; PA, phosphatidic acid; PE: phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; NL, sum of neutral lipids; PL, sum of polar lipids; TL, sum of total lipids.

Lipid class	Shrimp (n = 4)	Mackerel (n = 5)	Sardine (n = 4)	Squid (n = 6)	Liver & squid (n = 9)
WE	80.9 ± 12.9 ^{b}	84.2 ± 14.9 ^{b}	114.1 ± 15.6 ^{ab}	134.7 ± 7.0 ^{a}	115.3 ± 23.2 ^{ab}
CE	9.5 ± 1.0 ^{b}	10.6 ± 3.0 ^{ab}	10.8 ± 0.9 ^{b}	13.9 ± 0.9 ^{a}	11.7 ± 1.5 ^{b}
TG	34.8 ± 5.4	34.6 ± 9.4	31.1 ± 2.7	37.1 ± 3.1	31.1 ± 6.6
DG	4.0 ± 0.1	3.5 ± 0.3	3.3 ± 0.2	3.6 ± 0.6	3.4 ± 0.2
MG	0.02 ± 0.00 ^{b}	0.03 ± 0.01 ^{ab}	0.03 ± 0.01 ^{ab}	0.03 ± 0.01 ^{a}	0.02 ± 0.01 ^{b}
PA	19.5 ± 0.3 ^{b}	19.2 ± 0.8 ^{b}	16.6 ± 0.8 ^{c}	19.0 ± 2.1 ^{bc}	25.2 ± 2.4 ^{a}
PE	2.4 ± 0.2 ^{ab}	2.7 ± 0.2 ^{a}	2.7 ± 0.1 ^{a}	2.4 ± 0.1 ^{b}	2.0 ± 0.1 ^{c}
PI	0.1 ± 0.0 ^{a}	0.0 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}	0.1 ± 0.1 ^{a}	0.0 ± 0.0 ^{b}
PC	30.0 ± 0.4 ^{b}	33.5 ± 1.8 ^{ab}	33.5 ± 1.0 ^{ab}	32.6 ± 2.7 ^{ab}	35.3 ± 2.1 ^{a}
SM	1.0 ± 0.1 ^{b}	1.3 ± 0.2 ^{ab}	1.1 ± 0.1 ^{ab}	1.4 ± 0.1 ^{a}	1.1 ± 0.1 ^{b}
NL	129.2 ± 14.7 ^{b}	133 ± 24.4 ^{b}	159.3 ± 15.1 ^{b}	189.3 ± 8.2 ^{a}	161.5 ± 29.4 ^{b}
PL	53.0 ± 0.5 ^{c}	56.7 ± 2.6 ^{b}	54.0 ± 1.8 ^{bc}	55.5 ± 4.7 ^{bc}	63.6 ± 4.3 ^{a}
TL	182.2 ± 15.0 ^{bc}	189.8 ± 22.4 ^{c}	213.3 ± 14.0 ^{bc}	244.8 ± 8.3 ^{a}	225.2 ± 29.0 ^{ab}

Table 5.2: Lipid class concentrations (mean ± 1 S.D. of spawns, mg lipid g^{-1} dw) in red drum larvae from different maternal diets at 21 dph in Experiment 1.

Boldface type indicates lipid class concentrations that differed significantly among diet groups (ANOVA, FDR = 0.05). For each lipid class, values that share the same superscript letter are not significantly different (Tukey, adjusted $P < 0.05$). Abbreviations for lipid classes can be found in Table 5.1 caption.

Lipid class	Shrimp (n = 4)	Mackerel (n = 5)	Sardine (n = 4)	Squid (n = 6)	Liver & squid (n = 9)
WE	5.2 ± 1.7 ^{ab}	5.8 ± 1.6 ^{ab}	3.8 ± 0.8 ^{b}	3.9 ± 0.3 ^{b}	6.0 ± 0.7 ^{a}
CE	5.5 ± 0.2	5.4 ± 1.1	5.1 ± 2.4	5.7 ± 0.6	6.1 ± 1.4
TG	28.3 ± 8.6 ^{a}	15.7 ± 2.5 ^{b}	20.9 ± 6.6 ^{ab}	17.7 ± 3.9 ^{b}	15.3 ± 2.6 ^{b}
DG	4.0 ± 0.2	4.3 ± 0.3	4.1 ± 0.3	4.1 ± 0.2	4.2 ± 0.3
MG	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
PA	0.8 ± 0.0 ^{a}	0.7 ± 0.0 ^{b}	0.7 ± 0.0 ^{b}	0.6 ± 0.0 ^{b}	0.6 ± 0.0 ^{b}
PE	15.7 ± 0.3	15.2 ± 0.7	15.4 ± 0.4	15.5 ± 0.5	15.4 ± 0.4
PI	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0
PS	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
PC	29.5 ± 0.8	29.9 ± 1.4	30.2 ± 0.7	30.5 ± 0.5	30.1 ± 1.0
SM	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.4 ± 0.0	1.5 ± 0.1
NL	43.2 ± 8.9 ^{a}	31.3 ± 4.1 ^{ab}	33.9 ± 9.5 ^{ab}	31.5 ± 4.0 ^{b}	31.8 ± 1.5 ^{b}
PL	47.8 ± 1.0	47.5 ± 2.1	48.1 ± 0.9	48.2 ± 0.8	47.9 ± 1.4
TL	91.0 ± 9.0 ^{a}	78.8 ± 3.1 ^{b}	82.0 ± 9.6 ^{ab}	79.7 ± 4.5 ^{b}	79.7 ± 2.0 ^{b}

Table 5.3: Principal component loadings (with varimax rotation) for fatty acid concentrations (mg g^{-1} dw) of red drum larvae at 21 dph reared from eggs produced by adult fish fed different diets in Experiment 1 shown in Figure 5.2a. Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	**-0.84**	0.07
15:0	0.38	0.05
16:0	**0.85**	-0.16
16:1n-7	-0.56	-0.29
16:2n-4	-0.23	0.43
17:0	**0.80**	-0.18
16:3n-4	0.55	0.19
18:0	**0.92**	-0.15
18:1n-9	0.48	**-0.73**
18:1n-7	0.68	-0.48
18:2n-6	-0.44	**-0.84**
18:3n-6	**-0.77**	0.19
18:3n-4	0.24	0.64
18:3n-3	**-0.90**	-0.21
18:4n-3	**-0.92**	-0.04
20:1n-9	-0.08	0.49
20:2n-6	-0.34	-0.33
20:3n-6	-0.45	-0.58
20:4n-6	**0.82**	0.26
20:3n-3	-0.59	-0.39
20:4n-3	**-0.83**	-0.43
20:5n-3	-0.09	0.32
22:1n-11	-0.16	0.36
22:4n-6	**0.89**	-0.22
22:5n-6	0.19	**0.80**
22:5n-3	0.59	-0.34
22:6n-3	0.19	**0.77**

Table 5.4: Lipid class concentrations (mg lipid g^{-1} dw, mean ± 1 S.D.) of red drum eggs from different broodstock tanks and maternal diets in Experiment 2.

Boldface type indicates lipid class concentrations that differ significantly among diet groups. Asterisks indicate significant differences between larvae from different broodstock tanks (two-way ANOVA, $P < 0.05$). There was no significant interaction between tank and diet. Abbreviations for lipid classes can be found in Table 5.1 caption.

Lipid class	H3		H4		MT7	
	Shrimp	Fish	Shrimp	Fish	Shrimp	Fish
WE/SE	69.3 ± 8.9	66.2 ± 9.4	63.6 ± 6.7	54.7 ± 15.0	76.7 ± 2.6	58.9 ± 4.5
TG	63.2 ± 13.6	72.7 ± 11.2	62.4 ± 4.5	62.1 ± 3.5	69.8 ± 4.9	66.0 ± 8.0
ST	8.3 ± 1.4	8.6 ± 0.5	10.3 ± 1.2	8.7 ± 1.3	9.2 ± 1.2	9.5 ± 1.1
PE*	15.1 ± 0.8	14.7 ± 1.5	9.7 ± 4.8	12.0 ± 1.8	15.0 ± 2.1	13.4 ± 2.0
PI/PA*	3.9 ± 0.3	4.3 ± 0.2	2.4 ± 1.3	3.6 ± 0.6	4.2 ± 0.4	4.1 ± 0.5
PS*	2.7 ± 0.2	2.8 ± 0.1	1.6 ± 0.6	2.2 ± 0.6	2.8 ± 0.5	2.8 ± 0.3
PC*	55.9 ± 4.9	58.4 ± 2.8	44.0 ± 17.7	46.9 ± 5.4	53.5 ± 7.9	55.1 ± 4.5
NL	140.7 ± 22.2	147.5 ± 20.2	136.2 ± 10.4	125.4 ± 18.8	155.7 ± 4.3	134.4 ± 10.3
PL*	77.6 ± 5.8	80.3 ± 4.6	57.6 ± 24.1	64.7 ± 8.0	75.4 ± 10.0	75.3 ± 7.0
TL*	218.3 ± 27.1	227.8 ± 15.6	193.8 ± 18.0	190.1 ± 20.8	231.1 ± 10.0	209.8 ± 15.5

Table 5.5: Lipid class concentrations (mg lipid g^{-1} dw; mean ± 1 S.D.) of red drum larvae from different broodstock tanks and maternal diets at 21 dph in Experimenet 2.

Asterisks indicate significant differences between larvae from different broodstock tanks (two-way ANOVA, $P < 0.05$). There was no significant interaction between tank and diet. Abbreviations for lipid classes can be found in Table 5.1 caption.

Lipid class	H3		H4		MT7	
	Shrimp	Fish	Shrimp	Fish	Shrimp	Fish
TG*	20.2 ± 6.4	17.8 ± 11.4	34.9 ± 15.4	31.6 ± 9.0	19.1 ± 9.4	18.5 ± 8.3
ST	12.7 ± 0.8	12.4 ± 1.3	12.9 ± 1.2	12.3 ± 0.8	11.4 ± 1.3	11.9 ± 2.7
PE	20.4 ± 1.8	23.9 ± 3.8	18.9 ± 2.9	21.9 ± 3.0	23.4 ± 3.0	20.9 ± 3.3
PI/PA	3.3 ± 0.4	3.7 ± 0.6	3.2 ± 0.4	4.0 ± 0.7	3.5 ± 0.6	3.2 ± 0.3
PS	10.3 ± 1.2	10.9 ± 1.6	8.6 ± 1.9	10.6 ± 1.5	10.2 ± 1.9	9.9 ± 2.0
PC	46.4 ± 2.4	52.2 ± 14.8	48.0 ± 4.3	53.8 ± 7.4	49.1 ± 5.2	47.2 ± 6.6
NL*	32.9 ± 7.0	30.2 ± 12.7	47.8 ± 14.3	44.0 ± 8.4	30.5 ± 9.6	30.4 ± 9.0
PL	80.3 ± 5.3	90.6 ± 20.8	78.6 ± 9.3	90.2 ± 12.3	86.2 ± 9.4	81.3 ± 10.8
TL*	113.2 ± 7.0	120.8 ± 33.4	126.4 ± 6.8	134.2 ± 11.9	116.7 ± 13.7	111.7 ± 16.9

Table 5.6: Principal component loadings for fatty acid profiles (mg g^{-1} dw; PQN and scaled) in total lipids of red drum larvae at 21 dph reared from eggs produced by adult fish fed different diets in Experiment 2 shown in Figure 5.5. Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	0.42	0.69
15:0	**0.73**	0.16
16:0	**0.90**	0.12
16:1n-7	-0.47	0.58
16:2n-4	-0.30	**-0.80**
17:0	0.58	0.23
16:3n-4	-0.05	-0.50
18:0	0.53	-0.57
18:1n-9	-0.53	0.51
18:1n-7	-0.45	0.54
18:2n-6	**-0.98**	0.03
18:3n-6	0.35	0.22
18:3n-4	0.47	-0.20
18:3n-3	**-0.72**	0.67
18:4n-3	-0.36	**0.85**
20:1n-9	-0.41	**-0.76**
20:2n-6	-0.23	-0.24
20:3n-6	0.03	-0.63
20:4n-6	**0.95**	0.02
20:3n-3	**-0.89**	0.15
20:4n-3	**-0.92**	-0.04
20:5n-3	-0.09	0.23
22:1n-11	0.63	0.00
22:4n-6	0.31	**-0.73**
22:5n-6	**0.87**	0.43
22:5n-3	-0.32	**-0.80**
22:6n-3	**0.88**	0.40

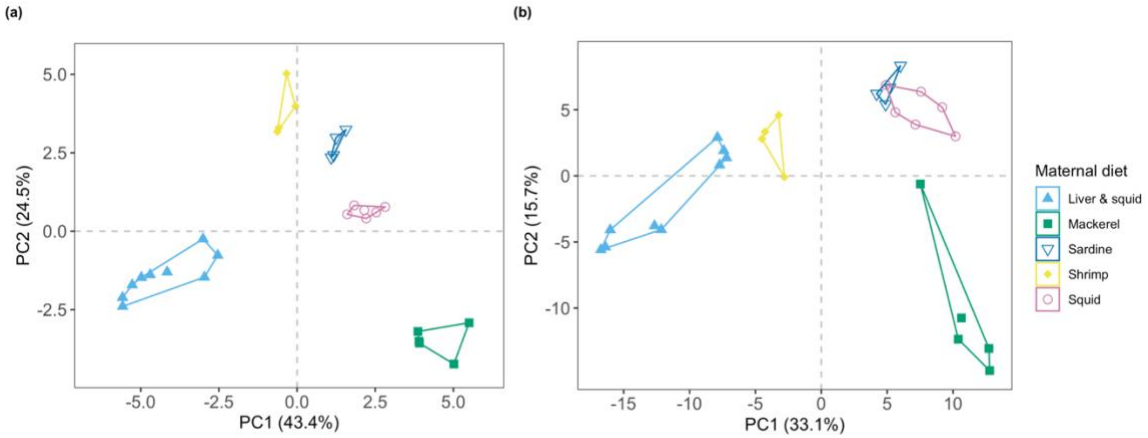

Figure 5.1: Principal component analysis of (a) fatty acid concentrations (mg g^{-1} dw) and (b) lipidomic profiles (peak intensities) of red drum eggs produced by adult fish fed different diets in Experiment 1.

Principal component loadings are in Table D3 and Figure D1 respectively. Each point represents a spawn. Colors and symbols indicate diet groups (blue filled triangle: liver & squid diet; green square: mackerel diet; dark blue open triangle: sardine; yellow diamond: shrimp diet; pink circle: squid diet).

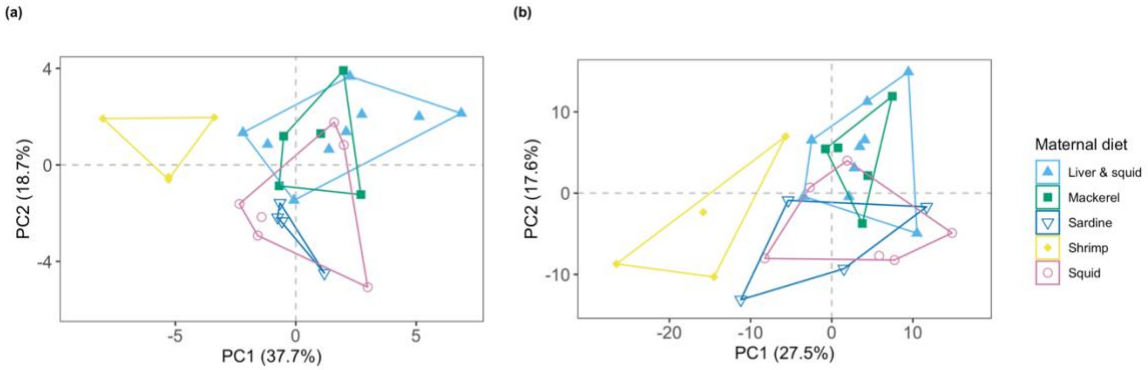

Figure 5.2: Principal component scores (with varimax rotation) of (a) fatty acid profiles (mg g^{-1} dw) and (b) lipidomic profiles (peak intensities) of red drum larvae at 21 dph reared from eggs produced by adult fish fed different diets in Experiment 1 (data were PQN and scaled).

Principal component loadings are in Table 5.3 and Figure D2 respectively. Each point represents larvae reared from a spawn. Colors and symbols indicate diet groups (blue filled triangle: liver & squid diet; green square: mackerel diet; dark blue open triangle: sardine; yellow diamond: shrimp diet; pink circle: squid diet).

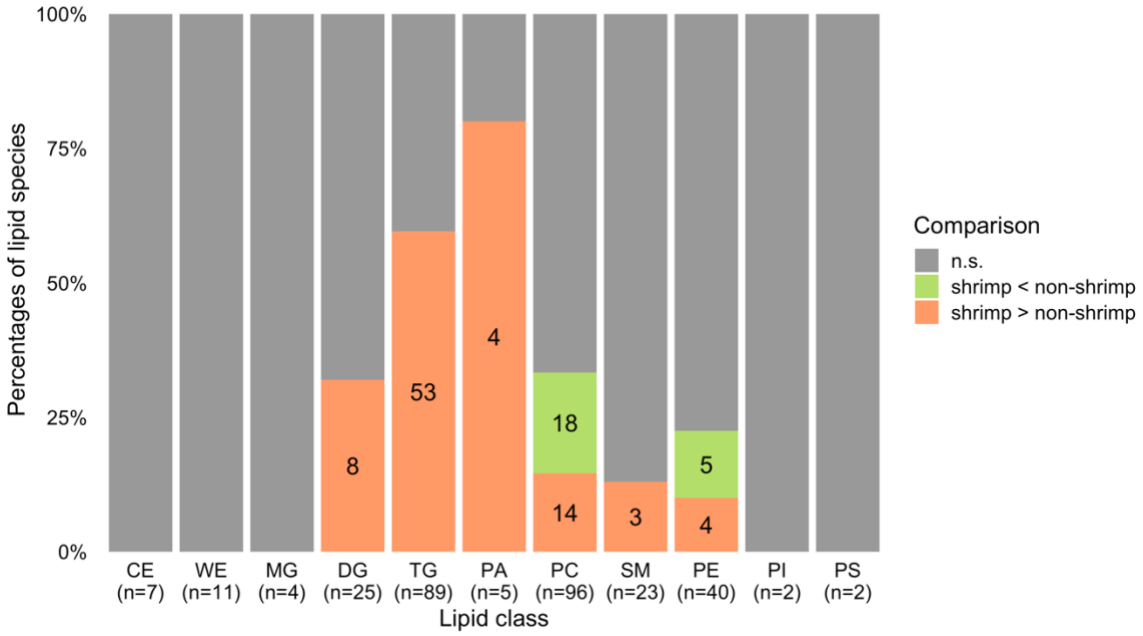

Figure 5.3: Percentages of lipid species that were significantly different between larvae from the shrimp diet and non-shrimp diet groups, by lipid class in Experiment 1.

Colors inidicate direction of difference (green: shrimp < non-shrimp; orange: shrimp > non-shrimp; gray: not significantly different (n.s.)). Total number of identified lipid species (n) is indicated in the parentheses. Total number of significant lipid species is indicated on the bars. Lipid classes: CE, cholesteryl ester; WE, wax ester; MG, monoglyceride; DG, diglyceride; TG, triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; SM, sphingomyelin; PE: phosphatidylethanolamine; PI, phosphatidylinositol; PS: phosphatidylserine.

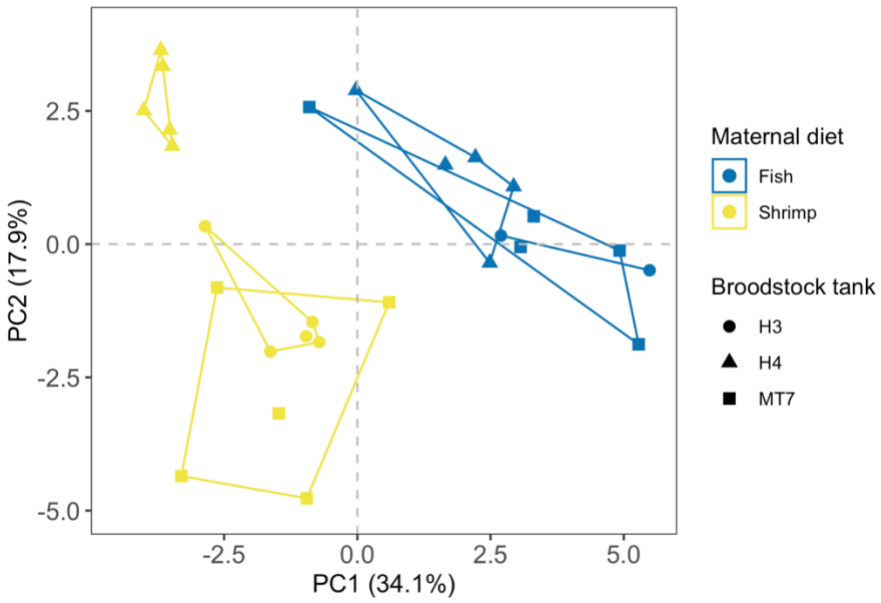

Figure 5.4: Principal component analysis (with varimax rotation) of fatty acid concentrations (mg g^{-1} dw) of red drum eggs produced by adult fish fed different diets in Experiment 2.

Principal component loadings are in Table D4. Each point represents a spawn. Colors indicate diet groups (dark blue: fish diet; yellow: shrimp diet). Symbols indicate broodstock tanks (circle: H3; triangle: H4; square: MT7).

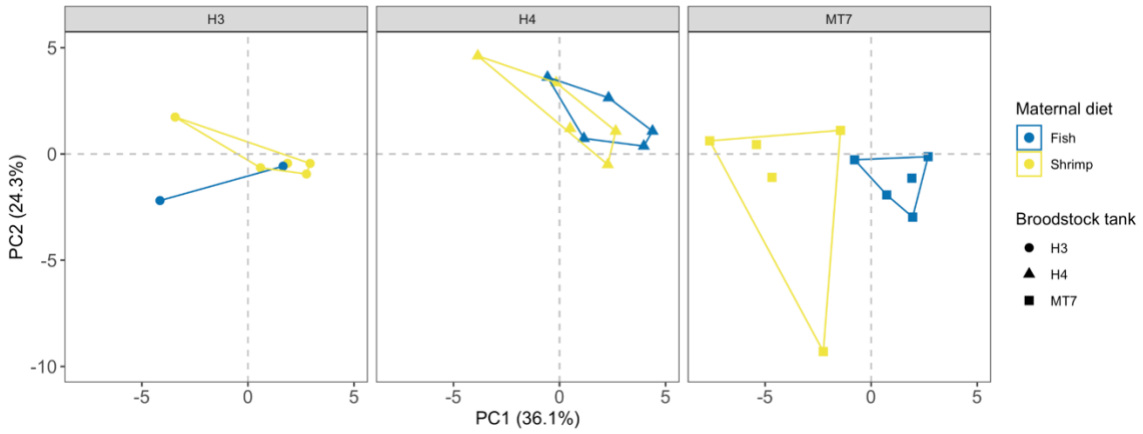

Figure 5.5: Principal component analysis of fatty acid profiles (mg g^{-1} dw; PQN and scaled) in total lipids of 21-dph red drum larvae produced by adult fish fed different diets from Experiment 2.

Principal component loadings are in Table 5.6. Each point represents larvae reared from a spawn. Colors indicate diet groups (dark blue: fish diet; yellow: shrimp diet). Symbols indicate broodstock tanks (circle: H3; triangle: H4; square: MT7).

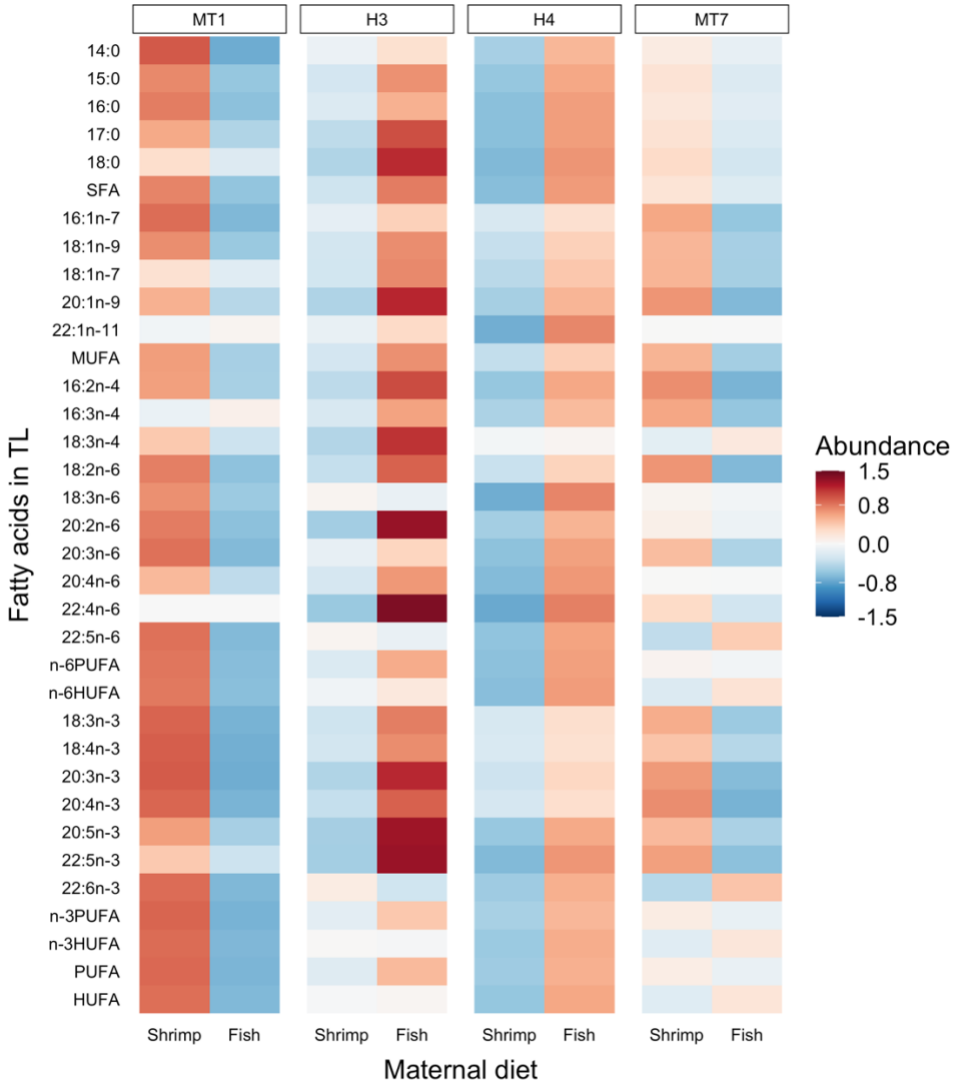

Figure 5.6: Heat map showing concentrations of fatty acids (mg g^{-1} dw) in total lipids of red drum larvae at 21 dph produced by adult fish (MT1 from Experiment 1, and H3, H4, MT7 from Experiment 2) fed shrimp or fish diets.

Color of each cell indicates fatty acid concentrations, which were scaled (mean = 0, S.D. = 1) separately for each broodstock tank to highlight maternal dietary effect. Columns represent diet groups from each broodstock tank. Rows represent an individual fatty acid or their group sums.

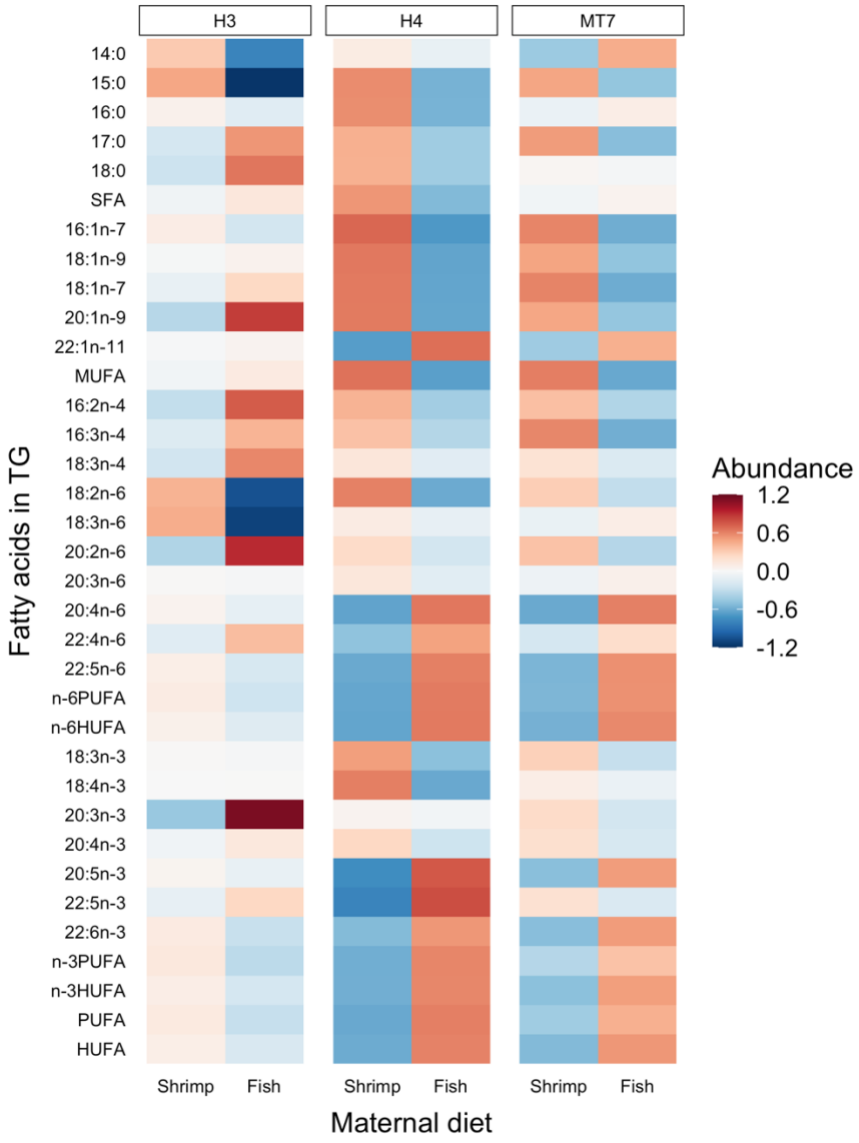

Figure 5.7: Heat map showing abundance of fatty acids (% total fatty acids) in TG of red drum larvae at 21 dph produced by adult fish from Experiment 2.

Color of each cell indicates fatty acid percentages, which were scaled (mean = 0, S.D. = 1) separately for each broodstock tank to highlight maternal dietary effect. Columns represent diet groups from each broodstock tank. Rows represent individual Rows represent individual fatty acids or their group sums.

Chapter 6: Conclusions

This dissertation addresses the parent–progeny relationship in the context of lipid nutrition and metabolism. The research investigated the process of maternal–offspring transfer of lipids and constituent fatty acids, and the transgenerational effects of varying maternal nutrition on offspring utilization of endogenous (maternally-derived) and exogenous (larval diet) lipids. The study species, red drum (*Sciaenops ocellatus*), is a batch-spawning fish that produces millions of eggs during multiple spawning events within a reproductive season. Considering the high reproductive output, they are likely to rely heavily on recent dietary intake of fatty acids, rather than previously accumulated body stores, as a source of nutrients that are deposited in eggs. The degree to which egg fatty acid profiles reflect maternal dietary intake of fatty acids was investigated in Chapter 2 using a series of diet-shift experiments. Fifteen individual fatty acids and nine sums or ratios of fatty acids in red drum eggs responded predictably to variations in maternal diet within 2.5-17 days. Results suggested little modification of polyunsaturated fatty acids between ingestion and incorporation into oocytes, but greater modification of some saturated and monounsaturated fatty acids, likely via biosynthesis and/or oxidation. In addition, the rate of incorporation of dietary fatty acids into eggs was proportional to the magnitude of change in dietary intake for 11 fatty acids, suggesting rapid changes in egg fatty acid concentrations following drastic diet shifts.

Results from Chapter 3 took a step further and provided a complete assessment of the maternal dietary effect on egg lipid composition. In contrast to the drastic changes in egg fatty acid compositions after diet shifts, egg lipid class profiles remained relatively stable with only subtle differences in the concentrations of several lipid classes, despite substantial differences in those of maternal diets. Variations were greater for neutral lipid

classes (wax ester/steryl ester, triglyceride, sterol) than for polar lipid classes, with variations in egg triglyceride content being driven by triglyceride content of maternal diets. In addition, fatty acids from maternal diets were incorporated into all three major lipid classes (triglyceride, wax ester/steryl ester, phosphatidylcholine) in eggs, but the diet-induced variations in fatty acid profiles were greater in neutral lipid classes than the polar lipid class. This study provides insight into the pathways of maternal-offspring nutrient transfer, which are likely different for neutral and polar lipids.

These two studies (Chapters 2 and 3) suggest a degree of maternal control over the provisioning of lipids as structural components (phospholipids) and energy substrates (neutral lipids), which may ensure proper development of larvae, but they also highlight the contribution of maternal diet to variations in egg composition – an important determinant of embryonic and early larval growth and development. It will be interesting to investigate if there is a "threshold" for female control over egg composition, especially under conditions such as a dietary deficiency of important nutrients, and if and to what extent females utilize nutrients from body stores to maintain the "minimum" egg composition under such circumstances. It may also be worthwhile to explore the possibility that other important nutrients, such as indispensable amino acids, transfer from maternal diet to eggs. Such information will provide a holistic view of the maternal dietary effects on embryonic nutrition. In addition, the strong diet-egg correlations for fatty acids (particularly those in neutral lipids) suggest that the fish eggs, especially eggs of income breeders, may be a useful source material for studying adult fish diet. This information may be of interest to ecologists who wish to study aquatic food web dynamics.

The consequences of varying egg lipid compositions (in response to variations in maternal diets) for larval physiology and performance during the endogenous and exogenous feeding period were examined in the following chapters. Results from Chapter

4 showed that embryos and larvae used 15 fatty acids from the yolk and oil globule at rates that differed among parental diet groups and were proportional to the initial concentrations of those fatty acids in the eggs. This resulted in diminishing differences in larval fatty acid compositions among maternal diet groups over time. Nevertheless, larval fatty acid composition remained different among parental diet groups at the time of onset of exogenous feeding and afterward (until death by starvation). These results suggest that the differences in fatty acid composition of yolk and oil changed the substrates that were available to embryos and early larvae for both energy production and building new tissues. Such altered energy resources and tissue composition may lead to changes in larval membrane biophysical properties, physiological processes, and behavioral performances, such as predator escape responses. Further studies are needed to characterize the scope and severity of the consequences for offspring.

Additionally, rates of utilization of the oil globule (neutral lipids) were different among diet groups, resulting in differences in larval oil globule size at the first-feeding stage. The differences in how long the oil globule lasts might be associated with differences in egg TG concentration resulting from the maternal diet (reported in Chapter 3). A larger oil globule at first feeding increases a larva's chances of surviving by improving its ability to withstand starvation. These results implicate effects of maternal nutrition in larval survival and critical physiological functions during the critical period of transition to exogenous feeding.

Chapter 5 addressed the longer-term effect of maternal nutrition on larval lipid metabolism. Larvae reared from distinct egg profiles showed differences in total fatty acid accumulation and retention of highly unsaturated fatty acids (especially in triglyceride) at 21 days post-hatching. Altered larval fatty acid composition could have consequences for ecologically relevant performance (growth, survival, swimming, and predator escape). The

results presented in this chapter raised some interesting questions that could be further explored in future studies. The observed changes in larval lipid and fatty acid composition could derive from altered ingestion, digestion, absorption, biosynthesis, or oxidation of fatty acids by larvae. More studies are needed to examine the key genes and/or enzymes involved in these lipid homeostatic pathways, especially on the organ level, to clarify the physiological mechanisms responsible for the differences in larval composition. Dietary fatty acid retention and the metabolic fate can be determined using tracer studies (e.g., radioactive label). Furthermore, results from this chapter showed that the variations in larval lipid metabolism induced by maternal diet also varied according to females. This "female effect" could be explained by the female genetics (e.g., gene polymorphism), or non-genetic traits that include but are not limited to size, age, condition, and physiological state (e.g., hormone levels). Future studies should better control for or address these factors by incorporating them into statistical models for data analysis. Molecular approaches, such as transcriptomics, may provide insight into the role played by female genotypes. In addition, more studies are needed to identify the nutrient component(s), present or absent in the shrimp diet, that cause nutritional programming. It is possible that non-lipid nutritional constituents (e.g., amino acids, vitamins) in the eggs are also influenced by changes in maternal diet. Metabolomic studies that characterize a range of metabolites may help identify the nutritional stimulus. Future studies should better control for the concurrent variations in many nutritional components in the maternal diets by using formulated diet, or microinjection into the fertilized eggs. The challenge for those studies is to identify the nutrient that should be manipulated.

This research shows that maternal nutrition can have profound transgenerational effects on offspring physiology. Female red drum quickly transfer dietary lipids and fatty acids into developing oocytes. Variations in these maternally derived nutrients can lead to

altered utilization of endogenous and exogenous lipids by offspring, giving rise to differences in larval body composition. The altered lipid metabolism may have consequences for larval physiological processes and behavioral performance, which may ultimately influence larval survival and fitness. Such transgenerational effects may be ecologically relevant because changes in adult diet occur in nature during spawning migration and as a result of changes in the food web dynamics that are associated with climate change, shifts in habitat and ecosystem structure.

Appendices

APPENDIX A. DYNAMICS OF DIET-EGG TRANSFER OF FATTY ACIDS IN THE TELEOST FISH, RED DRUM (*SCIAENOPS OCELLATUS*)

Supplementary Materials and Methods

Broodstock care

Adult red drum (length: 90 – 100 cm, weight: 9 – 15 kg) were maintained at the Fisheries and Mariculture Laboratory (FAML) of the University of Texas Marine Science Institute in Port Aransas, TX, and the Texas Parks and Wildlife (TPWD) CCA Marine Development Center in Corpus Christi, TX. Adults were collected from nearby waters at least 1 year prior to each diet change and presumed to be genetically unrelated. Fish were held in 12,000-16,000 L recirculating tanks and were induced to spawn naturally under a controlled temperature (24-26°C) and photoperiod (10:14 L:D) regime at a salinity of 30-38 ppt and 6.15-7.40 mg L^{-1} dissolved oxygen.

Diet components were previously frozen shrimp (*Farfantepenaeus aztecus* or *Litopenaeus setiferus*), Spanish sardine (*Sardinella aurita*), Atlantic mackerel (*Scomber scombrus*), and squid (*Loligo opalescens*). In some experiments, diets were supplemented with capsules containing Algamac ARA (Alg ARA) and Algamac 3050 (Alg 3050), which have high levels of ARA (20:4n-6) and DHA (22:6n-3), respectively. These products are commercial live prey enrichments derived from spray-dried cells of *Cyrothecodinium* and heterotrophically grown *Schizochytrium* sp. algae, respectively (Aquafauna Bio-Marine Inc, Hawthorne, CA, USA). Soy lecithin (phophatidylcholine) was purchased from MilliporeSigma (St. Louis, MO, USA), and was high in 18:2n-6. Algamac ARA, Algamac 3050, and soy lecithin were fed to the fish in gelatin capsules that were placed inside whole

shrimp or squid. Vitalis CAL is a pelleted marine fish feed (Skretting, St. Andrews, NB, CA).

Fish were hand fed several pieces of one prey type at a time that were spread around the tank so that, as much as possible, all broodstock received similar amounts of food. Feeding was immediately stopped when the designed ration was reached or when fish stopped eating to make sure there were no uneaten food items in the tanks. The weight of unconsumed food, if any, was taken into account when recording the ingested amount for that day. A daily feeding record is provided as a separate file (Supplementary Document D1).

Lag analysis

Lag analyses were conducted on data obtained from five broodstock tanks that were given multiple diet shifts in sequence. Details of the multiple-diet-shift experiments are shown in Table A1. Egg samples were collected whenever fish spawned, approximately every 2-6 days. Spawns sampled for these experiments are shown in a separate file (Supplementary Document D2).

The recorded amounts of food ingested at each meal were used to calculate a time series of daily intake for each tank, by calculating a moving average of dietary FA intake per fish for the seven preceding days. The time series of daily intake started 1 month before the first spawn sampled (24 days for MT1 and MT7) and ended on the date of the last spawn sampled.

For each FA in each experiment, the Pearson correlation coefficient, r, was calculated between the amount of that FA in the eggs and the mean daily intake of that FA on the days of the spawns sampled (lag = 0). Correlation coefficients were also calculated between the FA in eggs and mean daily intake on each day prior to the spawn dates up to

30 days (lag = 1-30). A correlogram was constructed for each FA in each experiment to ensure there was a progressive trend in correlations (i.e., maximum *r* was not random) (Figure A1) and the maximum *r* was used to estimate the lag between ingestion and incorporation into eggs. When the maximum *r* did not fall along the trendline in its correlogram (i.e., random error), the lag was designated as the next highest *r* on the trendline. This occurred only once: the second highest *r* ($r = 0.75$, at a lag of 7 days) was designated for 16:1n-7/16:0 in MT7 because the maximum *r* ($r = 0.79$) at a lag of 20 days was not on the trendline.

Incorporation rate analysis

Table A2 summarizes details of the 21 single-diet-shift experiments. Each diet shift represented a different amount of change in dietary intake for each FA. The simple dilution model predicts that turnover (or incorporation) rate of a FA is proportional to the magnitude of change in dietary intake. The magnitude of change for each FA (ΔFA; mg d^{-1} $fish^{-1}$) was calculated as the difference between the 28-day mean of dietary intake after and 28-day mean of dietary intake before the diet shift.

Since spawning frequency could not be controlled, the number of and interval between egg samples obtained for each experiment varied (approximately every 2-8 days), resulting in inconsistent accuracy and precision of estimates of incorporation rate.

For three FA in experiment 17 (18:2n-6, 18:3n-6, and 18:3-4), the slopes of the linear regression between egg FA level and the time since a diet change were considered inaccurate estimates of incorporation rates and were excluded from the analysis because the levels of these FA in eggs stabilized after the first spawn following the shift. Other estimates were deemed inaccurate because the standard error of those slopes (I_{FA}) were

large (> 4 standard deviations above the mean of all standard errors in the data set for that FA; Table A3).

Linear regressions between I_{FA} and ΔFA were used to test the prediction of the simple dilution model (Figure 2.4).

Biochemical analysis

The amount of FA in the eggs and dietary items was measured by gas chromatography using established methods (Faulk and Holt 2005). Briefly, lyophilized samples were homogenized and lipids were extracted with 2:1 chloroform: methanol (v/v). A known amount of tricosanoic acid (23:0) (Supelco, Inc., Bellefonte, PA, USA) was added before homogenization as an internal standard. Fatty acid methyl esters (FAME) were prepared by saponification in potassium hydroxide in methanol and transesterification with 14% boron trifluoride in methanol. FAME were dissolved in hexane before analysis by gas chromatography. Samples collected in 2010 (exp 2) were analyzed on a Hewlett-Packard 5890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a Supelcowax 10 fused silica capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Supelco, Inc.). All other samples were analyzed on a Shimadzu GC-2014 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with an FID and a Phenomenex ZB-WAX plus capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Phenomenx, Torrance, CA, USA) or a Supelcowax 10 column. FAME were identified by comparison with commercial standards.

Supplementary Results

Prey composition

Feed items varied greatly in FA profile. FA profile for the same feed item sometimes varied over the years. A full FA profile for prey items can be found in a separate file Supplementary Document D3.

Table A1: Summary of diet-shift experiments for lag analysis.

Each row indicates the sequence and duration (days) of each diet.

Tank	Date of		Number of spawns	Diet change	
	first spawn	last spawn		Diets	Duration
MT1	11 Mar 2018	12 Sept 2018	34	shrimp, liver	34
				shrimp, squid	35
				shrimp, sardine	57
				mackerel	78
				shrimp, squid, sardine	6
MT6	24 Mar 2012	19 Jun 2012	23	shrimp, squid, sardine	33
				squid, shrimp, sardine, 5 g Alg ARA	58
				shrimp	26
MT7	11 Mar 2018	8 Jun 2018	17	sardine, squid	34
				sardine	35
				squid	45
MT8	27 Oct 2011	5 Jan 2012	27	shrimp, squid	13
				shrimp, squid, mackerel	36
				shrimp	52
MT9	24 Feb 2012	19 Sept 2012	32	shrimp	31
				shrimp, squid	56
				shrimp	112
				shrimp, squid, sardine	41

Table A2: Summary of single-diet-shift experiments.

An arrow separates the diet before the diet shift from the diet after the shift. Experiments using VB and MT tanks were conducted at TPWD and FAML, respectively. Commercial products included in some diets were Algamac ARA (Alg ARA), Algamac 3050 (Alg 3050), soy lecithin, and Vitalis CAL.

Expt.	Diet change	Date of diet shift	Tank	Number of		
				fish	females	spawns
1	shrimp, mackerel, squid → shrimp, squid, 1.8 g Alg ARA	9 Jun 2010	VB07	2	1	13
2	shrimp, squid → shrimp, squid, 1.3 g Alg ARA	20 Jun 2011	VB07	2	1	8
3	shrimp, squid, 1.3 g Alg ARA → shrimp, squid, 3.8 Alg ARA	5 Aug 2011	VB07	2	1	4
4	squid, shrimp, sardine → squid, shrimp, sardine, 5 g Alg ARA	28 Mar 2012	MT6	4	1	12
5	squid, shrimp, sardine, 5 g Alg ARA → shrimp	25 May 2012	MT6	4	1	7
6	shrimp, squid → shrimp, squid, mackerel	10 Oct 2011	MT8	2	1	5
7	shrimp, squid, mackerel → shrimp	15 Nov 2011	MT8	2	1	15
8	shrimp → shrimp, squid	24 Feb 2012	MT9	2	1	8
9	shrimp, squid → shrimp	20 Apr 2012	MT9	2	1	7
10	shrimp → shrimp, squid, sardine	10 Aug 2012	MT9	2	1	10
11	shrimp, squid, liver, mackerel → shrimp, liver	19 Jul 2012	VB3-1	5	3	9
12	shrimp, squid, liver, mackerel → shrimp, squid, mackerel	3 Sept 2012	VB4-1	5	3	7
13	shrimp, squid, sardine → shrimp	15 Aug 2012	MT7	4	2	7
14	shrimp, 4.9 g Alg 3050 → shrimp, squid	23 Jan 2012	MT8	2	1	9
15	shrimp → Vitalis CAL	8 Jul 2013	VB3-3	5	3	14

16	shrimp, sardine, squid, 3.1 g soy lecithin → shrimp, sardine, squid	25 Aug 2014	MT9	3	1	11
17	shrimp, liver → shrimp, squid	21 Mar 2018	MT1	4	2	10
18	shrimp, squid → shrimp, sardine	25 Apr 2018	MT1	4	2	4
19	shrimp, sardine → mackerel	21 Jun 2018	MT1	4	2	4
20	sardine, squid → sardine	21 Mar 2018	MT7	4	2	4
21	sardine → squid	25 Apr 2018	MT7	4	2	5

Table A3: Inaccurate estimates of I_{FA}, as determined by large standard errors for the slope (> 4 standard deviations above the mean of all standard errors).

Fatty acid	Experiment
14:0	19
15:0	6
16:0	3, 6, 9, 12
ΣSAT	6, 9
18:1n-7	5
18:1n-9	12
20:1n-9	19
22:1n-11	19
ΣMUFA	12
16:2n-4	6
16:3n-4	9
18:3n-4	6, 17
18:2n-6	17
18:3n-6	6, 17
20:2n-6	6
20:3n-6	11
20:4n-6	5
Σ(n-6)PUFA	11
18:4n-3	19
20:4n-3	19
22:5n-3	6
22:6n-3	9
ΣPUFA	9
ΣHUFA	9
Σ(n-3) PUFA	9
Σ(n-3) HUFA	9
ΣC16/ΣC18	9
Σ(n-3)/Σ(n-6) PUFA	9
Σ(n-3)/Σ(n-6) HUFA	14, 21

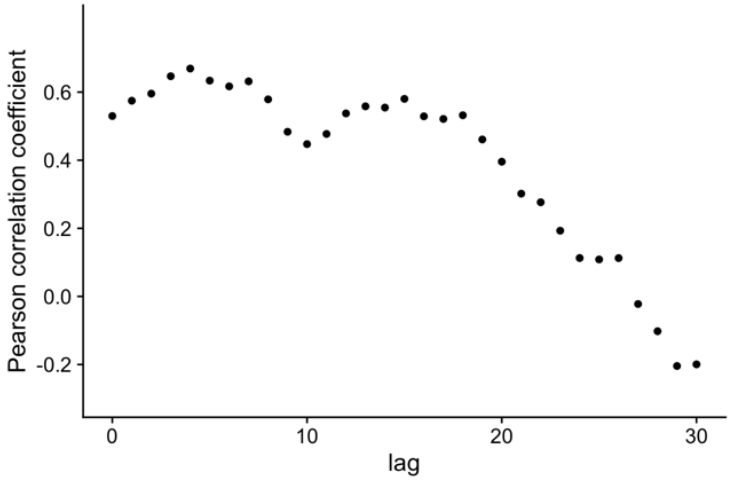

Figure A1: Sample correlogram showing the strength of the relationship (expressed as *r*) between egg DHA content and daily DHA dietary intake for lags of 0-30 days in tank MT1. Here, the maximum *r* (0.67) corresponds to a lag of 4 days.

APPENDIX B. INCORPORATION OF DIETARY LIPIDS AND FATTY ACIDS INTO RED DRUM *SCIAENOPS OCELLATUS* EGGS

Supplementary Materials and Methods

Table B1: Mean lipid intake (g fish^{-1} week^{-1}) for broodstock tanks.

Mean lipid intake (g fish^{-1} week^{-1}) for broodstock tanks, calculated from the mean weekly dietary intake (g wet weight tank^{-1} week^{-1}), diet item dry weight (dw) to weight wet (ww) ratio, number of fish in the tank, and total lipid content of diet items (mg g^{-1} dw; Table 3.2).

Broodstock diet	Broodstock tank	Diet item	Weekly intake	dw/ww ratio	No. fish	Lipid intake
Shrimp	MT1	Shrimp	7667	0.24	4	17.7
Mackerel	MT1	Mackerel	3146	0.28	4	35.7
Squid	MT7	Squid	6767	0.23	4	45.1
Liver & squid	MT7	Liver	1913	0.24	4	15.8
		Squid	1900	0.23	4	12.7
Herring	MT7	Herring	3000	0.28	4	18.9
Sardine	H4	Sardine	3120	0.29	3	26.0
	MT1		3000	0.29	4	18.7

Table B2: Quaternary gradient mobile phase composition for neutral lipid separation and quantification.

A, isooctane: ethyl acetate (99.8:0.2, v/v); B, methanol: water (90:10, v/v); C, acetone: ethyl acetate (2:1, v/v); D, ethyl acetate. Flow rate = 0.8 ml/min. Modified from Abreu et al. (2017).

Time	Percent solvent			
(min)	A	B	C	D
0	100	0	0	0
1.5	100	0	0	0
1.5	97	0	3	0
9	94	0	6	0
11	70	0	30	0
14	45	0	55	0
15	45	0	55	0
16	40	5	55	0
20	35	10	55	0
20	33	17	50	0
25	38	17	45	0
25	48	17	35	0
30	53	17	30	0
40	40	60	0	0
40	0	0	100	0
42	0	0	100	0
42	50	0	0	50
50	50	0	0	50
50	100	0	0	0
63	100	0	0	0

Table B3: Tertiary gradient mobile phase composition for polar lipid separation and quantification.

A, 0.5% diethylamine-formate, pH 3.0; B, isopropanol; C, isooctane. Flow rate = 0.3 ml/min. Modified from Plante et al. (2016).

Time	Percent solvent		
(min)	A	B	C
0	1	35	64
2	4	35	61
8	10	30	60
28	10	30	60
29	1	35	64
38	1	35	64

Supplementary Results

Diet fatty acid profiles

Table B4: Fatty acid composition (% total fatty acid, mean ± 1 S.D.) in total lipids of different types of diet items.

Fatty acid	Diet item					
	Mackerel	Sardine	Herring	Squid	Liver	Shrimp
14:0	6.5 ± 0.7	5.6 ± 0.4	5.1 ± 1.1	3.6 ± 0.4	0.7 ± 0.2	1.8 ± 0.2
15:0	0.5 ± 0.0	1.1 ± 0.0	1.0 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	1.3 ± 0.2
16:0	15.1 ± 1.5	24.3 ± 0.4	20.0 ± 1.1	24.2 ± 0.7	13.7 ± 2.3	14.0 ± 0.4
16:1n-7	3.2 ± 0.4	6.5 ± 0.2	5.1 ± 1.2	2.2 ± 0.3	0.7 ± 0.3	4.3 ± 0.5
16:2n-4	0.9 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.1
17:0	0.3 ± 0.0	1.6 ± 0.1	1.3 ± 0.2	0.3 ± 0.1	0.7 ± 0.1	2.0 ± 0.1
16:3n-4	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	1.5 ± 0.6
18:0	3.0 ± 0.5	7.3 ± 0.1	6.2 ± 0.7	2.6 ± 0.3	28.0 ± 3.2	9.3 ± 0.5
18:1n-9	11.5 ± 3.1	6.3 ± 0.7	5.4 ± 1.1	10.7 ± 1.4	13.3 ± 3.5	7.5 ± 0.9
18:1n-7	2.2 ± 0.3	2.7 ± 0.2	3.0 ± 0.2	4.7 ± 0.5	1.1 ± 0.3	3.9 ± 0.5
18:2n-6	1.5 ± 0.2	1.9 ± 0.2	1.7 ± 0.1	0.8 ± 0.1	12.0 ± 3.9	2.2 ± 0.5
18:3n-6	0.1 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
18:3n-4	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1
18:3n-3	0.7 ± 0.2	0.8 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.5 ± 0.4	0.6 ± 0.1
18:4n-3	2.1 ± 0.6	0.7 ± 0.1	0.9 ± 0.3	1.0 ± 0.2	0.0 ± 0.0	0.2 ± 0.0
20:1n-9	10.0 ± 1.9	0.4 ± 0.1	0.4 ± 0.1	2.7 ± 0.8	0.2 ± 0.1	0.7 ± 0.3
20:2n-6	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	1.1 ± 0.2
20:3n-6	0.2 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	5.0 ± 1.1	0.5 ± 0.2
20:4n-6	0.7 ± 0.2	2.6 ± 0.1	3.3 ± 0.2	1.1 ± 0.2	10.2 ± 2.7	6.1 ± 0.5
20:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1
20:4n-3	0.6 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0.3	0.5 ± 0.1
20:5n-3	4.4 ± 0.6	6.6 ± 0.4	8.1 ± 1.7	13.7 ± 0.5	0.5 ± 0.2	14.2 ± 1.5
22:1n-11	20.9 ± 3.4	0.1 ± 0.0	0.1 ± 0.0	1.7 ± 0.4	0.1 ± 0.1	0.4 ± 0.2
22:4n-6	0.1 ± 0.0	0.5 ± 0.1	1.0 ± 0.3	0.1 ± 0.0	2.0 ± 0.3	0.8 ± 0.2
22:5n-6	0.2 ± 0.1	1.4 ± 0.0	1.8 ± 0.3	0.2 ± 0.0	0.5 ± 0.2	0.8 ± 0.1
22:5n-3	0.9 ± 0.2	1.0 ± 0.0	1.6 ± 0.2	0.6 ± 0.1	2.9 ± 1.2	1.5 ± 0.4
22:6n-3	8.9 ± 1.8	18.3 ± 0.7	23.4 ± 3.2	21.2 ± 2.7	1.1 ± 0.2	11.4 ± 1.5
Total identified	95.3 ± 0.7	93.7 ± 0.5	94.2 ± 0.4	95.5 ± 0.3	94.9 ± 1.0	87.8 ± 1.6

Table B5: Principal component loadings for total lipid fatty acid composition of different diet items shown in Figure 3.2a.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	**0.74**	0.27
15:0	0.56	-0.68
16:0	0.68	-0.09
16:1n-7	**0.73**	-0.45
16:2n-4	**0.87**	0.14
17:0	0.19	**-0.87**
16:3n-4	0.25	-0.47
18:0	**-0.96**	-0.25
18:1n-9	-0.54	0.59
18:1n-7	**0.75**	-0.01
18:2n-6	**-0.92**	-0.10
18:3n-6	0.20	**-0.85**
18:3n-4	0.56	-0.55
18:3n-3	0.52	-0.10
18:4n-3	0.52	0.64
20:1n-9	0.18	**0.85**
20:2n-6	0.35	-0.33
20:3n-6	**-0.95**	-0.12
20:4n-6	**-0.87**	-0.41
20:3n-3	0.53	0.28
20:4n-3	0.43	0.45
20:5n-3	**0.73**	-0.13
22:1n-11	0.11	**0.79**
22:4n-6	**-0.82**	-0.45
22:5n-6	0.30	**-0.79**
22:5n-3	-0.69	-0.26
22:6n-3	**0.82**	-0.28

Table B6: Principal component loadings for diet item fatty acid content (% total fatty acids) in major lipid classes (triglyceride, free fatty acid, phosphatidylethanolamine, phosphatidylcholine) shown in Figure 3.3.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2	PC3
14:0	**-0.63**	0.14	-0.51
15:0	-0.52	0.27	0.25
16:0	-0.24	**-0.70**	0.20
16:1n-7	**-0.68**	0.47	-0.08
16:2n-4	**-0.64**	0.22	-0.17
17:0	0.00	0.19	0.26
16:3n-4	-0.10	-0.18	0.45
18:0	**0.94**	0.10	-0.09
18:1n-9	0.29	-0.38	-0.26
18:1n-7	-0.51	0.28	0.10
18:2n-6	**0.81**	0.23	-0.21
18:3n-6	-0.02	0.49	0.08
18:3n-4	-0.34	-0.18	0.34
18:3n-3	-0.41	**0.61**	-0.32
18:4n-3	-0.41	0.43	-0.54
20:1n-9	-0.43	-0.25	**-0.70**
20:2n-6	-0.17	0.28	0.40
20:3n-6	**0.81**	0.31	-0.23
20:4n-6	**0.74**	0.43	0.07
20:3n-3	-0.21	0.16	0.21
20:4n-3	-0.24	**0.64**	-0.19
20:5n-3	-0.55	0.47	0.33
22:1n-11	-0.31	-0.18	**-0.75**
22:4n-6	**0.83**	0.38	0.02
22:5n-6	-0.05	0.44	0.43
22:5n-3	0.57	**0.65**	-0.08
22:6n-3	-0.51	0.08	0.43

Egg fatty acid profiles

Table B7: Fatty acid composition (% total fatty acid, mean ± 1 S.D.) in total lipids of eggs from different diet groups.

Fatty acid	Diet group					
	Mackerel	Sardine	Herring	Squid	Liver & squid	Shrimp
14:0	5.0 ± 0.3	2.3 ± 0.4	2.2 ± 0.6	2.4 ± 0.4	1.1 ± 0.2	1.2 ± 0.1
15:0	0.5 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
16:0	20.3 ± 0.6	24.9 ± 1.3	23.4 ± 0.4	25.8 ± 1.9	24.4 ± 1.2	26.5 ± 1.0
16:1n-7	5.1 ± 0.1	7.8 ± 1.0	7.6 ± 0.6	6.0 ± 0.5	6.3 ± 1.0	12.3 ± 1.6
16:2n-4	1.1 ± 0.0	1.3 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	0.3 ± 0.0
17:0	0.3 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.8 ± 0.0
16:3n-4	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	1.0 ± 0.1
18:0	3.9 ± 0.2	4.9 ± 0.2	4.7 ± 0.1	3.9 ± 0.4	8.6 ± 1.1	5.0 ± 0.3
18:1n-9	18.4 ± 0.6	12.1 ± 1.3	9.6 ± 3.4	12.1 ± 1.0	13.4 ± 0.9	15.2 ± 1.2
18:1n-7	2.4 ± 0.2	2.8 ± 0.2	4.2 ± 2.9	2.9 ± 0.6	1.4 ± 0.3	3.5 ± 0.1
18:2n-6	1.8 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	0.9 ± 0.0	8.7 ± 2.0	1.0 ± 0.2
18:3n-6	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3n-4	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3n-3	0.9 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
18:4n-3	1.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.0
20:1n-9	3.5 ± 0.4	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.0
20:2n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
20:3n-6	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	2.0 ± 0.5	0.2 ± 0.0
20:4n-6	1.1 ± 0.1	2.7 ± 0.1	3.2 ± 0.3	1.5 ± 0.2	6.1 ± 0.9	3.3 ± 0.3
20:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4n-3	1.2 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
20:5n-3	4.9 ± 0.4	3.3 ± 0.6	4.0 ± 0.3	7.1 ± 0.9	2.4 ± 0.7	3.3 ± 0.5
22:1n-11	2.7 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:4n-6	0.4 ± 0.0	0.9 ± 0.2	0.9 ± 0.0	0.4 ± 0.1	2.1 ± 0.3	0.9 ± 0.3
22:5n-6	0.4 ± 0.0	1.4 ± 0.2	1.2 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	0.7 ± 0.1
22:5n-3	2.1 ± 0.1	2.3 ± 0.1	2.5 ± 0.4	2.5 ± 0.2	2.6 ± 0.2	2.8 ± 0.3
22:6n-3	16.2 ± 0.3	23.6 ± 1.2	25.5 ± 0.8	24.9 ± 2.2	13.4 ± 2.9	14.1 ± 2.3
Total identified	94.8 ± 0.5	96.5 ± 0.6	96.5 ± 0.2	96.0 ± 0.4	96.4 ± 0.2	94.0 ± 1.8

Table B8: Principal component loadings for total lipid fatty acid composition of eggs from different diet groups shown in Figure 3.2b.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	**0.84**	0.42
15:0	0.55	**-0.71**
16:0	-0.42	-0.52
16:1n-7	-0.21	-0.66
16:2n-4	**0.76**	-0.16
17:0	0.00	**-0.90**
16:3n-4	0.18	**-0.72**
18:0	**-0.90**	0.29
18:1n-9	0.12	0.53
18:1n-7	0.35	-0.53
18:2n-6	**-0.79**	0.49
18:3n-6	0.08	-0.53
18:3n-4	0.29	-0.65
18:3n-3	**0.86**	0.31
18:4n-3	**0.78**	0.54
20:1n-9	0.61	0.67
20:2n-6	-0.63	0.64
20:3n-6	**-0.82**	0.45
20:4n-6	**-0.94**	0.09
20:3n-3	0.28	0.33
20:4n-3	**0.80**	0.56
20:5n-3	0.69	0.15
22:1n-11	0.60	0.64
22:4n-6	**-0.90**	0.19
22:5n-6	0.14	**-0.74**
22:5n-3	-0.53	-0.30
22:6n-3	0.56	-0.41

Table B9: Principal component loadings for total lipid fatty acid composition of eggs in major lipid classes (wax ester/steryl ester, triglyceride, phosphatidylcholine) shown in Figure 3.4.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	0.06	**-0.78**
15:0	-0.33	**-0.80**
16:0	**-0.79**	-0.49
16:1n-7	0.55	-0.15
16:2n-4	0.11	**-0.75**
17:0	-0.55	-0.30
16:3n-4	0.58	-0.25
18:0	**-0.72**	0.27
18:1n-9	**0.85**	0.12
18:1n-7	0.43	-0.62
18:2n-6	0.14	**0.72**
18:3n-6	**0.70**	-0.24
18:3n-4	0.63	0.09
18:3n-3	**0.91**	0.10
18:4n-3	**0.76**	-0.08
20:1n-9	0.44	-0.39
20:2n-6	0.28	0.58
20:3n-6	-0.11	**0.76**
20:4n-6	-0.38	**0.81**
20:3n-3	**0.73**	0.17
20:4n-3	**0.83**	0.06
20:5n-3	0.44	0.29
22:1n-11	0.34	-0.39
22:4n-6	-0.25	**0.82**
22:5n-6	-0.07	0.12
22:5n-3	0.60	0.58
22:6n-3	-0.22	0.07

APPENDIX C. MATERNAL DIET AFFECTS UTILIZATION OF ENDOGENOUS LIPIDS BY RED DRUM *SCIAENOPS OCELLATUS* EMBRYOS AND EARLY LARVAE

Supplementary Results

Fatty acid utilization (percentage data)

Despite the uniform decrease in concentrations (mg g^{-1} dw) of all fatty acids during development, there was selective retention of 18:0, 20:4n-6, 22:5n-6 and 22:6n-3 in all diet groups, as indicated by increases in the percentages (of total fatty acids) over time in all diet groups. There was selective catabolism of fatty acids 15:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:3n-3, 20:5n-3, 22:5n-3, as indicated by decreases in the percentages of these of fatty acids over time in all diet groups. Fatty acids 16:2n-4, 16:3n-4, 17:0, 18:3n-6, 18:3n-4, 20:1n-9, 20:2n-6 remained relatively constant (% total fatty acids; regression $P > 0.05$, except for 16:3n-4 in the full diet). Percentages of ΣMUFA decreased with time, whereas percentages of ΣSFA and ΣPUFA increased with time. The relative retention of SFA and PUFA (increases in percentages of ΣSFA and ΣPUFA over time) reflects their structural roles, as SFA and PUFA are usually esterified at the sn-1 and sn-2 positions of the glycerol backbone of the membrane phospholipids, respectively (Wiegand 1996; Mourente et al. 1999).

Significant differences in rates of utilization (% total fatty acids) were detected for 11 fatty acids: 14:0, 15:0, 16:0, 16:1n-7, 22:1n-11, 18:2n-6, 20:3n-6, 20:4n-6, 18:3n-3, 20:4n-3 and 20:5n-3 (mixed ANOVA interaction term, FDR = 0.05; Table C6) and Σn-6 PUFA. Generally, these fatty acids showed greater reductions when their initial levels were higher, and smaller reductions or retention when initial levels were lower. Percentages of 14:0, 18:3n-3, 20:4n3, and 22:1n-11 were highest in the mackerel diet group and decreased at a significantly faster rate in that group than in other diet groups (Table C6). Similarly, percentages of 18:2n-6 and 20:3n-6 were greatest and decreased significantly faster in the

liver & squid diet group, while remaining constant or decreasing slightly in the other diet groups (Table C6). Interestingly, the percentage of 20:4n-6 was greatest in the liver & squid diet group, and still increased significantly faster than the mackerel diet group. The percentage of 16:1n-7 decreased at a significantly faster rate in the shrimp diet group than the mackerel diet group (Table C6). The mackerel diet group had the lowest percentage of 16:0 in the eggs, which then increased over time, whereas the percentages of 16:0 decreased in other diet groups (Table C6).

Table C1: Fatty acid profile (mg g^{-1} dw) of feed items.

	Diet item				
Fatty acid	Beef liver	Mackerel	Sardine	Shrimp	Squid
14:0	0.97 ± 0.21	13.14 ± 5.33	3.7 ± 0.32	0.64 ± 0.16	4.68 ± 2.05
15:0	0.31 ± 0.04	0.93 ± 0.38	1.33 ± 0.09	0.45 ± 0.13	0.51 ± 0.15
16:0	19.2 ± 3.31	29.53 ± 10.12	23.51 ± 4.01	4.88 ± 0.79	31.03 ± 12.53
16:1n-7	1.24 ± 0.27	6.54 ± 2.76	2.63 ± 0.38	1.49 ± 0.25	2.51 ± 1.72
16:2n-4	0.32 ± 0.08	1.72 ± 0.59	0.83 ± 0.06	0.14 ± 0.05	1.27 ± 0.9
17:0	1.05 ± 0.17	0.54 ± 0.19	1.3 ± 0.11	0.71 ± 0.14	0.47 ± 0.08
16:3n-4	0.58 ± 0.14	0.95 ± 0.37	0.35 ± 0.12	0.49 ± 0.15	0.39 ± 0.22
18:0	30.86 ± 1.36	5.77 ± 1.97	6.13 ± 0.91	3.23 ± 0.63	3.82 ± 0.58
18:1n-9	20.27 ± 4.34	23.33 ± 12.64	6.58 ± 1.87	2.6 ± 0.35	12.12 ± 8.55
18:1n-7	1.37 ± 0.23	4.27 ± 1.85	2.03 ± 0.29	1.37 ± 0.34	5.42 ± 3.57
18:2n-6	9.59 ± 0.62	2.96 ± 1.14	0.89 ± 0.1	0.76 ± 0.23	0.91 ± 0.56
18:3n-6	0.26 ± 0.09	0.12 ± 0.1	0.46 ± 0.03	0.15 ± 0.03	0.11 ± 0.07
18:3n-4	0.05 ± 0.01	0.1 ± 0.04	0.04 ± 0.03	0.06 ± 0.03	0.19 ± 0.11
18:3n-3	1.08 ± 0.27	1.49 ± 0.79	0.3 ± 0.04	0.2 ± 0.05	0.84 ± 0.62
18:4n-3	0.04 ± 0.01	4.26 ± 2.52	0.2 ± 0.01	0.06 ± 0.02	1.07 ± 0.82
20:1n-9	0.26 ± 0.09	20.97 ± 10.23	0.25 ± 0.09	0.25 ± 0.12	3.38 ± 1.86
20:2n-6	0.15 ± 0.02	0.52 ± 0.21	0.16 ± 0.03	0.35 ± 0.04	0.42 ± 0.17
20:3n-6	6.07 ± 0.4	0.32 ± 0.12	0.22 ± 0.04	0.17 ± 0.07	0.19 ± 0.11
20:4n-6	9.79 ± 0.74	1.2 ± 0.19	1.09 ± 0.09	2.17 ± 0.47	1.6 ± 0.3
20:3n-3	0.09 ± 0.02	0.28 ± 0.12	0.06 ± 0.02	0.09 ± 0.01	0.44 ± 0.15
20:4n-3	0.76 ± 0.17	1.13 ± 0.49	0.11 ± 0.02	0.16 ± 0.02	0.75 ± 0.52
20:5n-3	0.91 ± 0.18	8.84 ± 3.66	1.56 ± 0.37	4.83 ± 0.45	18.04 ± 6.34
22:1n-11	0.06 ± 0.06	42.35 ± 17.71	0.1 ± 0.09	0.14 ± 0.1	1.75 ± 1.39
22:4n-6	2.67 ± 0.26	0.26 ± 0.07	0.09 ± 0.02	0.29 ± 0.1	0.11 ± 0.04
22:5n-6	0.44 ± 0.04	0.36 ± 0.08	0.46 ± 0.05	0.3 ± 0.08	0.25 ± 0.06
22:5n-3	5.01 ± 0.9	1.82 ± 0.77	0.23 ± 0.07	0.54 ± 0.19	0.68 ± 0.32
22:6n-3	1.22 ± 0.15	16.97 ± 4.77	7.98 ± 0.12	3.9 ± 0.4	29.63 ± 5.62

Table C2: Principal component loadings for egg fatty acid composition.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2	PC3
14:0	**0.78**	-0.55	0.08
15:0	0.70	0.63	0.00
16:0	-0.23	**0.76**	0.22
16:1n-7	-0.01	**0.93**	-0.26
16:2n-4	**0.79**	0.00	0.53
17:0	0.10	**0.96**	-0.09
16:3n-4	0.45	**0.80**	-0.31
18:0	**-0.87**	-0.26	0.10
18:1n-9	0.59	-0.59	-0.29
18:1n-7	0.56	0.68	-0.33
18:2n-6	**-0.83**	-0.46	0.18
18:3n-6	0.04	0.59	0.45
18:3n-4	0.29	**0.78**	0.21
18:3n-3	**0.73**	-0.60	-0.10
20:1n-9	0.67	**-0.70**	-0.04
20:2n-6	-0.37	-0.64	0.16
20:3n-6	**-0.88**	-0.39	0.13
20:4n-6	**-0.96**	0.05	-0.07
20:3n-3	0.21	-0.05	-0.48
20:4n-3	**0.71**	-0.68	-0.09
20:5n-3	**0.85**	-0.10	0.14
22:1n-11	0.68	-0.69	-0.08
22:4n-6	**-0.95**	-0.17	0.02
22:5n-6	-0.01	**0.87**	0.02
22:5n-3	-0.42	0.25	**-0.71**
22:6n-3	0.43	0.47	0.63

Table C3: Loadings for principal components of larval fatty acid composition shown in Figure C1.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	-0.60	-0.66
15:0	**-0.93**	-0.13
16:0	**-0.96**	0.17
16:1n-7	**-0.90**	0.18
16:2n-4	-0.53	-0.61
17:0	**-0.75**	0.18
16:3n-4	**-0.82**	0.04
18:0	-0.13	**0.83**
18:1n-9	**-0.87**	-0.26
18:1n-7	**-0.92**	-0.02
18:2n-6	-0.21	0.58
18:3n-6	**-0.72**	-0.27
18:3n-4	**-0.77**	-0.38
18:3n-3	-0.64	-0.59
20:1n-9	-0.19	**-0.81**
20:2n-6	-0.59	0.38
20:3n-6	-0.12	0.67
20:4n-6	-0.19	**0.91**
20:3n-3	**-0.71**	-0.16
20:4n-3	-0.40	**-0.76**
20:5n-3	**-0.85**	-0.43
22:1n-11	-0.21	**-0.80**
22:4n-6	-0.07	**0.80**
22:5n-6	-0.53	0.21
22:5n-3	**-0.91**	0.19
22:6n-3	**-0.71**	-0.12

Table C4: Summary of mean fatty acid utilization rates (% initial level h^{-1}) during 36-84 hpf.

Values are means ± 1 S.D. of slopes of linear regressions performed for each spawn (n = 5) in each diet group. Boldface type indicates that utilization rates differ significantly among diet groups (mixed ANOVA interaction term, FDR = 0.05). For each fatty acid, slopes that share the same superscript letter are not significantly different (Tukey's HSD, adjusted $P < 0.05$).

Fatty acid	Diet group			
	Full	Shrimp	Mackerel	Liver & squid
Saturated fatty acids				
14:0	*-1.8* ± 0.4	*-2.1* ± 0.5	*-1.6* ± 0.4	*-1.7* ± 0.3
15:0	*-1.5* ± 0.4	*-1.6* ± 0.5	*-1.3* ± 0.3	*-1.4* ± 0.3
16:0	*-1.1* ± 0.3	*-1.2* ± 0.5	*-0.9* ± 0.2	*-1.1* ± 0.2
17:0	*-1.0* ± 0.3	*-1.0* ± 0.4	*-0.9* ± 0.3	*-1.1* ± 0.3
18:0	0.2 ± 0.1 [b]	0.2 ± 0.5 [b]	0.2 ± 0.1 [b]	*-0.4* ± 0.2 [a]
ΣSFA	*-1.0* ± 0.2	*-1.0* ± 0.4	*-0.9* ± 0.2	*-1.0* ± 0.2
Monounsaturated fatty acids				
16:1n-7	*-1.7* ± 0.4	*-1.8* ± 0.5	*-1.5* ± 0.3	*-1.7* ± 0.4
18:1n-7	*-1.3* ± 0.2	*-1.5* ± 0.5	*-1.5* ± 0.5	*-2.1* ± 1.2
18:1n-9	*-1.3* ± 0.3	*-1.4* ± 0.5	*-1.2* ± 0.2	*-1.3* ± 0.2
20:1n-9	*-1.3* ± 0.5	*-2.5* ± 1.1	*-1.8* ± 0.6	*-1.8* ± 0.9
22:1n-11	-1.2 ± 1.0	-2.6 ± 3.5	*-1.8* ± 0.3	*-3.7* ± 2.8
ΣMUFA	*-1.4* ± 0.3	*-1.6* ± 0.5	*-1.4* ± 0.2	*-1.5* ± 0.3
Polyunsaturated fatty acids				
n-4 fatty acids				
16:2n-4	*-1.3* ± 0.4	*-1.1* ± 0.4	*-1.2* ± 0.3	*-1.2* ± 0.4
16:3n-4	*-1.4* ± 0.6	*-1.5* ± 0.6	*-1.1* ± 0.2	*-0.8* ± 0.5
18:3n-4	*-1.0* ± 0.7	*-1.7* ± 0.7	*-3.5* ± 1.7	-1.6 ± 1.3
n-6 fatty acids				
18:2n-6	*-1.6* ± 0.6	*-1.5* ± 0.6	*-1.3* ± 0.2	*-1.6* ± 0.3

18:3n-6	-4.7 ± 4.8	-1.8 ± 2.3	*-4.4* ± 1.9	-2.5 ± 2.4
20:2n-6	-2.8 ± 2.6	-2.4 ± 2.3	*-1.2* ± 0.3	*-1.4* ± 0.5
20:3n-6	*-0.9* ± 0.3	*-1.7* ± 0.5	*-1.9* ± 0.6	*-1.4* ± 0.3
20:4n-6 (ARA)	-0.3 ± 0.3	*-0.4* ± 0.3	-0.1 ± 0.2	*-0.7* ± 0.2
22:4n-6	0.1 ± 0.5 [b]	-0.3 ± 0.5 [ab]	0.3 ± 1.0 [b]	*-1.0* ± 0.3 [a]
22:5n-6	*-0.7* ± 0.1	-0.3 ± 0.4	*-0.5* ± 0.4	-0.3 ± 0.6
Σn-6 PUFA	*-0.7* ± 0.2	*-0.6* ± 0.3	*-0.8* ± 0.2	*-1.2* ± 0.2
Σn-6 HUFA	*-0.3* ± 0.3	*-0.4* ± 0.3	-0.2 ± 0.3	*-0.9* ± 0.2
n-3 fatty acids				
18:3n-3	*-1.6* ± 0.7	*-1.5* ± 0.2	*-1.5* ± 0.3	*-1.6* ± 0.4
20:3n-3	*-3.5* ± 3.1	*-1.4* ± 1.5	*-1.9* ± 0.7	*-1.5* ± 0.7
20:4n-3	*-1.2* ± 0.4	*-0.8* ± 0.3	*-1.4* ± 0.2	*-1.7* ± 0.4
20:5n-3 (EPA)	*-1.4* ± 0.3	*-1.3* ± 0.3	*-1.2* ± 0.2	*-1.5* ± 0.3
22:5n-3	*-1.6* ± 0.3	*-1.3* ± 0.2	*-1.4* ± 0.2	*-1.5* ± 0.3
22:6n-3 (DHA)	*-0.8* ± 0.3	*-0.5* ± 0.1	*-0.6* ± 0.2	*-0.7* ± 0.3
Σn-3 PUFA	*-1.0* ± 0.3	*-0.8* ± 0.1	*-0.9* ± 0.2	*-1.0* ± 0.3
Σn-3 HUFA	*-1.0* ± 0.3	*-0.8* ± 0.1	*-0.9* ± 0.2	*-1.0* ± 0.3
ΣPUFA	*-1.0* ± 0.3	*-0.8* ± 0.2	*-0.9* ± 0.2	*-1.1* ± 0.2
ΣHUFA	*-0.9* ± 0.3	*-0.7* ± 0.1	*-0.8* ± 0.2	*-0.9* ± 0.2

Table C5: Summary of rates of change for fatty acid ratios (h^{-1}) during 36-84 hpf.

Values are means ± 1 S.D. of slopes of linear regressions performed for each spawn (n = 5) in each diet group. Boldface type indicates regression slopes that differ significantly among diet groups (mixed ANOVA interaction term, FDR = 0.05). For each fatty acid, slopes that share the same superscript letter are not significantly different (Tukey's HSD, adjusted $P < 0.05$).

Fatty acid ratio	Diet group			
	Full	Shrimp	Mackerel	Liver & squid
DHA:EPA	*0.059* ± 0.019 [b]	*0.063* ± 0.023 [b]	*0.038* ± 0.008 [b]	*0.126* ± 0.044 [a]
DHA:ARA	*-0.044* ± 0.009 [b]	-0.012 ± 0.013 [c]	*-0.063* ± 0.006 [a]	-0.001 ± 0.005 [cd]
EPA:ARA	*-0.017* ± 0.004 [b]	*-0.009* ± 0.002 [c]	*-0.036* ± 0.002 [a]	*-0.005* ± 0.001 [cd]
Σn-3:Σn-6 PUFA	*-0.018* ± 0.006 [a]	-0.008 ± 0.007 [b]	*-0.012* ± 0.004 [ab]	0.004 ± 0.003 [c]
Σn-3:Σn-6 HUFA	*-0.043* ± 0.005 [b]	-0.018 ± 0.008 [c]	*-0.067* ± 0.011 [a]	-0.002 ± 0.004 [d]

Table C6: Summary of fatty acid utilization rates (% total fatty acids h^{-1}) during 36-84 hpf.

Values are means ± 1 S.D. of slopes of linear regressions performed for each spawn (n = 5) in each diet group. Boldface type indicates regression slopes that differ significantly among diet groups (mixed ANOVA interaction term, FDR = 0.05). For each fatty acid, slopes that share the same superscript letter are not significantly different (Tukey's HSD, adjusted $P < 0.05$).

Fatty acid	Diet group			
	Full	Shrimp	Mackerel	Liver & squid
Saturated fatty acids				
14:0	*-2.21* ± 0.59 [b]	*-1.63* ± 0.66 [b]	*-4.93* ± 0.57 [a]	*-1.34* ± 0.19 [b]
15:0	*-0.41* ± 0.05 [a]	*-0.38* ± 0.14 [a]	*-0.19* ± 0.10 [b]	*-0.16* ± 0.05 [b]
16:0	-2.44 ± 2.34 [b]	-2.44 ± 1.99 [b]	3.62 ± 1.92 [a]	-1.57 ± 2.46 [b]
17:0	-0.004 ± 0.17	-0.04 ± 0.11	0.03 ± 0.19	-0.05 ± 0.15
18:0	*8.22* ± 1.61	*8.60* ± 2.49	*7.51* ± 0.94	*9.09* ± 1.67
ΣSFA	3.17 ± 3.70	2.28 ± 4.19	*6.05* ± 1.10	*5.97* ± 3.45
Monounsaturated fatty acids				
16:1n-7	*-8.73* ± 2.11 [ab]	*-11.91* ± 4.76 [a]	*-3.91* ± 0.60 [b]	*-7.40* ± 1.59 [ab]
18:1n-7	*-1.05* ± 0.78	*-1.48* ± 0.75	-1.50 ± 1.42	*-1.79* ± 1.79
18:1n-9	*-3.12* ± 0.76	*-4.46* ± 3.47	*-4.44* ± 1.88	*-4.84* ± 4.30
20:1n-9	0.04 ± 0.16	-0.12 ± 0.12	-0.86 ± 1.88	-0.09 ± 0.32
22:1n-11	-0.002 ± 0.07 [b]	-0.02 ± 0.10 [b]	*-2.47* ± 0.59 [a]	*-0.07* ± 0.04 [b]
ΣMUFA	*-12.87* ± 2.96	*-17.99* ± 8.99	*-13.19* ± 2.23	*-14.19* ± 5.48
Polyunsaturated fatty acids				
n-4 fatty acids				
16:2n-4	-0.07 ± 0.17	-0.01 ± 0.22	0.07 ± 0.38	0.07 ± 0.21
16:3n-4	-0.17 ± 0.20	*-0.39* ± 0.38	-0.03 ± 0.17	0.27 ± 0.44
18:3n-4	0.01 ± 0.06	-0.07 ± 0.04	-0.09 ± 0.05	-0.06 ± 0.10
n-6 fatty acids				
18:2n-6	-0.85 ± 0.42 [b]	*-0.59* ± 0.46 [b]	*-0.45* ± 0.17 [b]	*-6.54* ± 3.45 [a]
18:3n-6	-0.08 ± 0.09	0.06 ± 0.12	-0.04 ± 0.12	-0.01 ± 0.09
20:2n-6	0.05 ± 0.19	-0.002 ± 0.09	-0.01 ± 0.05	0.03 ± 0.06
20:3n-6	-0.002 ± 0.10 [b]	-0.11 ± 0.10 [b]	*-0.10* ± 0.06 [b]	*-1.11* ± 0.64 [a]

20:4n-6 (ARA)	*3.11* ± 0.67 [ab]	*3.97* ± 1.94 [ab]	*2.13* ± 0.33 [b]	*4.91* ± 1.63 [a]
22:4n-6	*1.02* ± 0.40	*0.79* ± 0.51	*0.67* ± 0.71	-0.001 ± 0.91
22:5n-6	*0.45* ± 0.08	*0.78* ± 0.35	*0.30* ± 0.28	*0.42* ± 0.09
Σn-6 PUFA	*3.71* ± 0.75 [b]	*4.89* ± 2.23 [b]	*2.49* ± 0.56 [b]	-2.30 ± 2.38 [a]
Σn-6 HUFA	*4.58* ± 1.01	*5.43* ± 2.59	*2.99* ± 0.76	4.22 ± 1.39
n-3 fatty acids				
18:3n-3	*-0.35* ± 0.24 [b]	*-0.28* ± 0.10 [b]	*-0.70* ± 0.11 [a]	*-0.31* ± 0.11 [b]
20:3n-3	*-0.10* ± 0.09	*-0.10* ± 0.13	*-0.17* ± 0.06	*-0.12* ± 0.09
20:4n-3	-0.08 ± 0.10 [bc]	0.04 ± 0.14 [c]	*-0.76* ± 0.13 [a]	*-0.27* ± 0.09 [b]
20:5n-3 (EPA)	*-1.99* ± 0.35 [a]	*-1.21* ± 0.29 [b]	*-1.08* ± 0.50 [b]	*-1.99* ± 0.36 [a]
22:5n-3	*-1.52* ± 0.49	*-1.05* ± 0.59	*-1.21* ± 0.49	*-1.68* ± 0.64
22:6n-3 (DHA)	*7.80* ± 3.16	11.45 ± 7.51	*11.69* ± 1.27	*14.05* ± 7.38
Σn-3 PUFA	*3.76* ± 3.64	8.85 ± 7.69	*7.77* ± 1.44	*9.69* ± 8.03
Σn-3 HUFA	*4.11* ± 3.61	9.13 ± 7.77	*8.47* ± 1.39	*10.00* ± 8.09
ΣPUFA	*7.25* ± 4.15	*13.28* ± 9.74	*10.21* ± 2.06	*7.68* ± 5.75
ΣHUFA	*8.70* ± 4.18	*14.55* ± 10.24	*11.46* ± 2.05	*14.21* ± 9.24

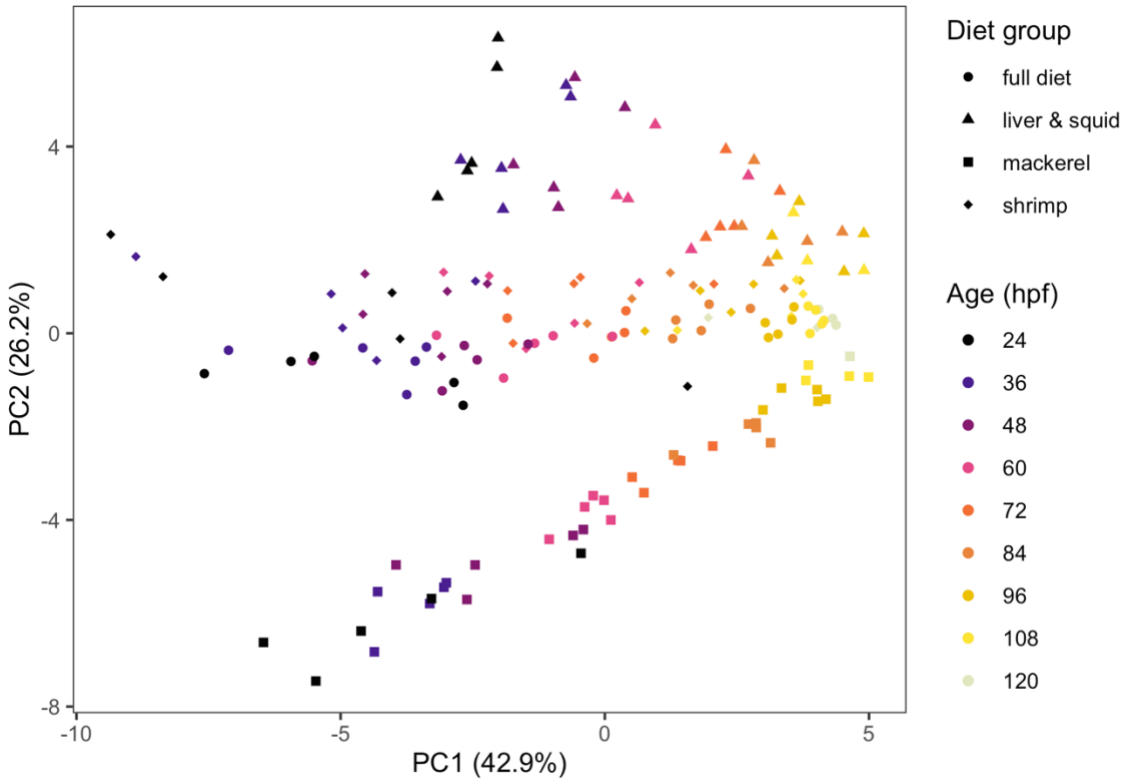

Figure C1: Principal components analysis (with varimax rotation) of concentrations of fatty acids (mg g^{-1} dw) in larval samples between 24-120 hpf, showing changes in fatty acid compositions of larvae from different diet groups over time.

Rotated principal components (PC1 and PC2) explained 42.9% and 26.2% of the total variance, respectively.

APPENDIX D. NUTRITIONAL PROGRAMMING BY MATERNAL NUTRITION ALTERS OFFSPRING LIPID METABOLISM

Supplementary Materials and Methods

Table D1: Summary of broodstock diet treatments and spawns in Experiment 1.

Broodstock Diet	Spawning Tank	No. of spawns	Spawn Date	Days on diet
Shrimp	MT1	4	1/21/18 – 1/31/18	> 1 yr
Sardine	MT7	4	4/2/18 – 4/26/18	12-36
Squid	MT7	6	4/29/18 – 6/8/18	5-45
Liver & squid	MT7	9	8/9/18 – 9/9/18	59-90
Mackerel	MT1	5	7/5/18 – 8/26/18	15-67

Table D2: Summary of broodstock diet treatments and spawns in Experiment 2.

Spawning Tank	Broodstock Diet	Spawn Date	Days on diet
H3	Shrimp	1/29/2020 – 2/12/2020	73-86
	Sardine	3/9/2020, 4/30/2020	14, 66
H4	Shrimp	1/15/2020 – 1/26/2020	71-82
	Sardine	4/5/2020 – 5/1/2020	41-67
MT7	Shrimp	2/29/2020 – 4/26/2020	25-82
	Herring	5/14/2020 – 5/27/2020	18-31

Supplementary Results

Experiment 1 – Egg lipid compositional profile

Table D3: Principal component loadings for fatty acid concentrations (mg g^{-1} dw) of eggs produced by adult fish fed different diets in Experiment 1 shown in Figure 5.1a. Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	**0.83**	0.49
15:0	0.64	-0.51
16:0	0.00	**-0.78**
16:1n-7	-0.05	**-0.83**
16:2n-4	**0.82**	0.09
17:0	-0.05	**-0.81**
16:3n-4	0.32	-0.60
18:0	**-0.92**	0.26
18:1n-9	0.49	0.46
18:1n-7	0.67	-0.61
18:2n-6	**-0.86**	0.45
18:3n-6	-0.43	-0.01
18:3n-4	0.31	-0.31
18:3n-3	**0.88**	0.35
18:4n-3	**0.77**	0.57
20:1n-9	0.62	0.68
20:2n-6	-0.62	0.68
20:3n-6	**-0.90**	0.40
20:4n-6	**-0.97**	0.08
20:3n-3	0.51	0.05
20:4n-3	**0.85**	0.50
20:5n-3	**0.80**	-0.08
22:1n-11	0.63	0.64
22:4n-6	**-0.94**	0.19
22:5n-6	0.04	-0.57
22:5n-3	-0.32	-0.46
22:6n-3	0.63	-0.29

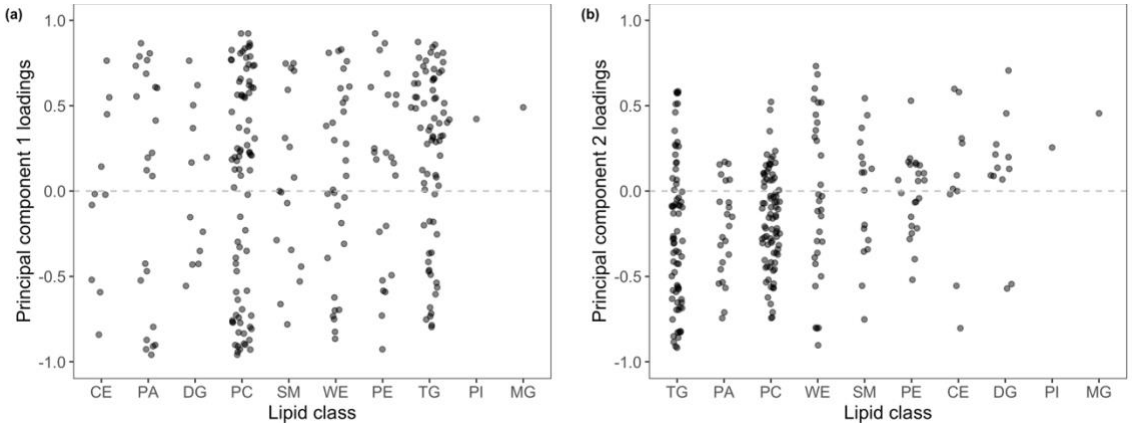

Figure D1: Principal component loadings for red drum egg lipidomic profile, shown in Figure 5.1b.

Lipid classes were ordered based on mean loadings (from left to right: lowest to highest mean loadings). Lipid classes: CE, cholesteryl ester; WE, wax ester; MG, monoglyceride; DG, diglyceride; TG, triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; SM, sphingomyelin; PE: phosphatidylethanolamine; PI, phosphatidylinositol.

Experiment 1 – Larval lipid compositional profile

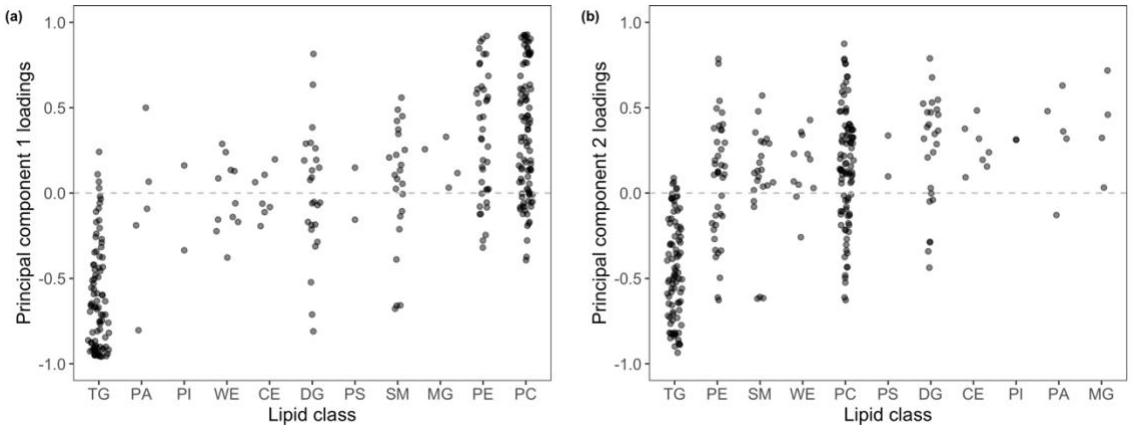

Figure D2: Principal component loadings for red drum larval lipidomic profile at 21 dph, shown in Figure 5.2b.

Lipid classes were ordered based on mean loadings (from left to right: lowest to highest mean loadings). Lipid classes: CE, cholesteryl ester; WE, wax ester; MG, monoglyceride; DG, diglyceride; TG, triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; SM, sphingomyelin; PE: phosphatidylethanolamine; PI, phosphatidylinositol; PS: phosphatidylserine.

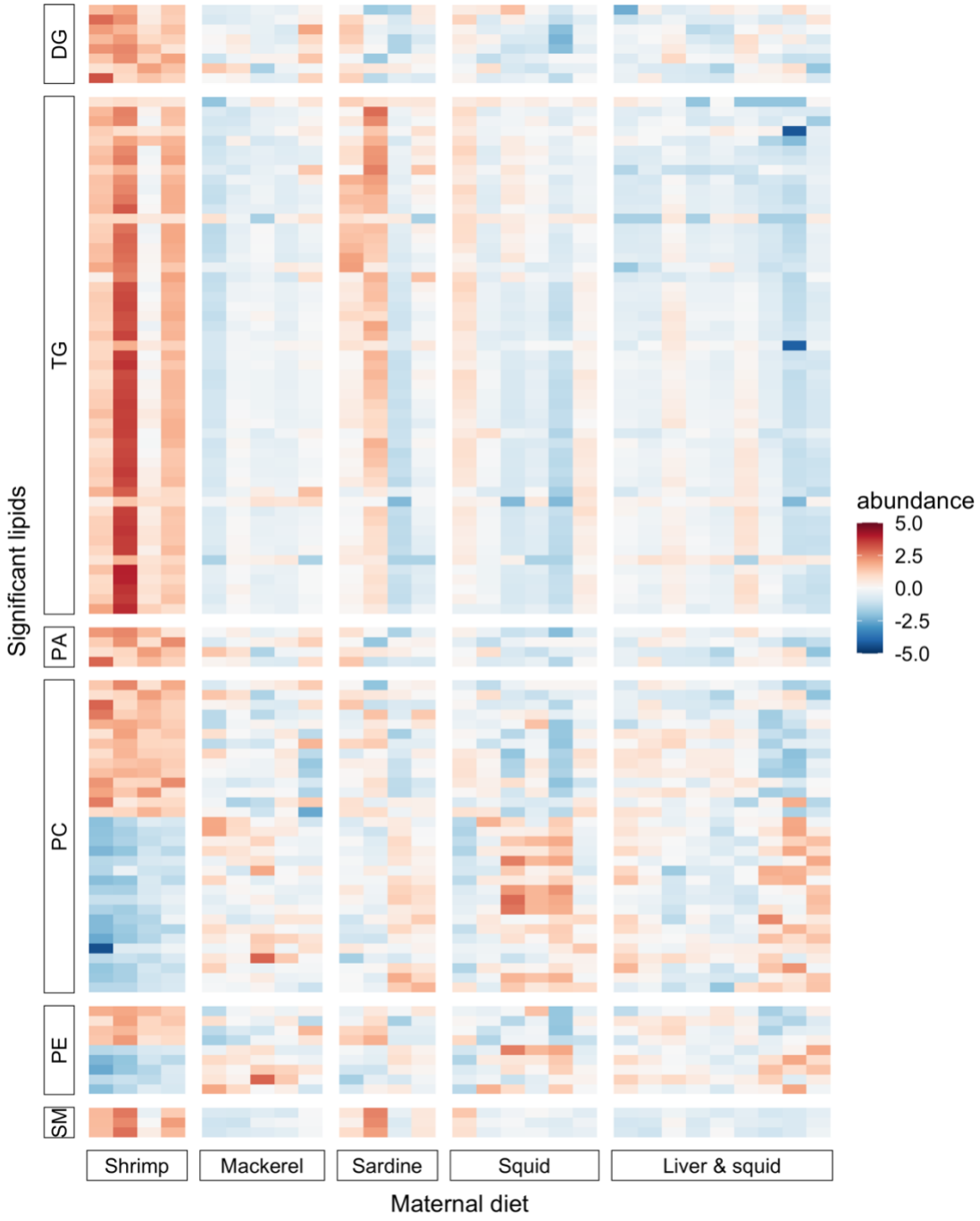

Figure D3: Heat map showing relative abundances of lipid species in larvae from Experiment 1.

Columns identify individual spawns, which are from diet groups of shrimp, mackerel (from MT1), sardine, squid, liver & squid (from MT7). Rows represent 109 lipid species from lipid classes (DG, diglyceride; TG, triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PE: phosphatidylethanolamine; SM, sphingomyelin) that were significantly different between larvae from the shrimp diet and non-shrimp diet groups. Color of each cell indicates the abundance of lipid species (peak intensity), which were normalized relative to sample dry weight, and scaled (mean = 0, sd = 1).

Experiment 2 – Egg lipid compositional profile

Fatty acid profiles of the neutral lipids (WE/SE, TG) in eggs also changed significantly after the diet shift (Figure D4; PERMANOVA pseudo-$F_{(1, 25)}$ = 7.3, 16.0, respectively; $P < 0.001$). Fatty acid profiles of the polar lipid, PC, were marginally significantly different between the two groups (Figure D3; PERMANOVA pseudo-$F_{(1, 25)}$ = 3.3; P = 0.053).

Table D4: Principal component loadings (with varimax rotation) for fatty acid concentrations (mg g^{-1} dw) of eggs produced by adult fish fed different diets in Experiment 2 shown in Figure 5.4.

Boldface type highlights strong loadings.

Fatty acid	PC1	PC2
14:0	**0.87**	-0.32
15:0	**0.82**	-0.22
16:0	-0.08	-0.45
16:1n-7	0.35	**-0.75**
16:2n-4	**0.76**	0.22
17:0	**0.74**	0.00
16:3n-4	-0.42	**-0.81**
18:0	0.52	0.24
18:1n-9	-0.57	-0.35
18:1n-7	0.68	-0.40
18:2n-6	0.37	0.35
18:3n-6	0.32	**-0.74**
18:3n-4	0.16	-0.12
18:3n-3	**0.94**	0.03
18:4n-3	**0.81**	0.12
20:1n-9	-0.61	-0.15
20:2n-6	0.10	-0.66
20:3n-6	-0.40	0.22

20:4n-6	-0.12	-0.26
20:3n-3	0.06	-0.48
20:4n-3	**0.88**	0.08
20:5n-3	0.66	-0.45
22:1n-11	-0.54	0.28
22:4n-6	0.54	-0.20
22:5n-6	**0.91**	0.21
22:5n-3	0.21	**-0.77**
22:6n-3	0.48	0.64

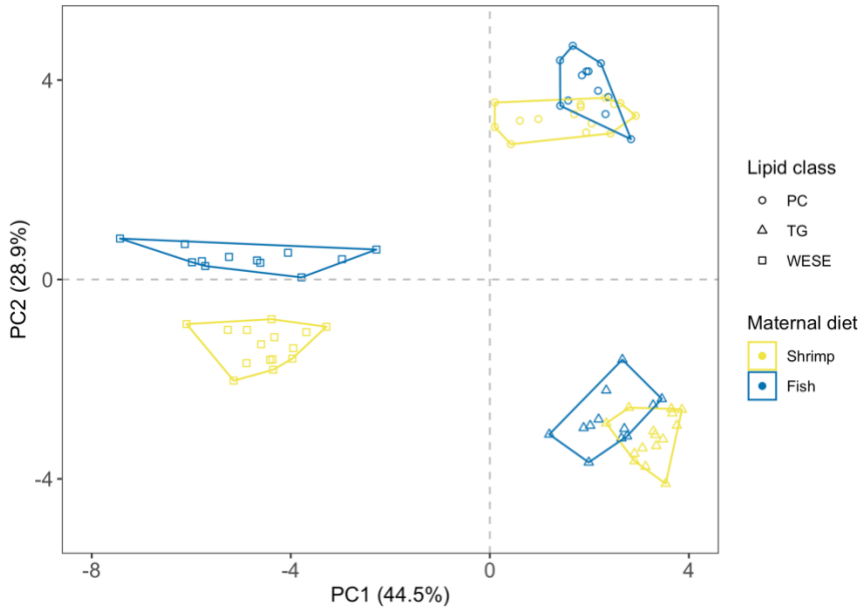

Figure D4: Principal component analysis of fatty acid profiles (% total fatty acids) in major lipid classes phosphatidylcholine (PC), triglyceride (TG), wax ester/steryl ester (WE/SE) of red drum eggs produced by adult fish fed different diets in Experiment 2.

Colors indicate diet groups (dark blue: fish diet; yellow: shrimp diet). Symbols indicate lipid classes (open circle: PC; open triangle: TG; open square: WE/SE).

Experiment 2 – Larval lipid compositional profile

Neutral lipid accumulation (particularly TG) with standard length differed due to female, not maternal diet. The slopes for TG over standard length was significantly higher in H4-shrimp than MT7-fish, and MT7-shrimp (Figure D5; Tukey adjusted $P < 0.05$). The slope of the regression of TG content (mg $larva^{-1}$) on larval standard length (mm) was greater for H4 (0.07 and 0.05 mg $larva^{-1}$ mm^{-1} for the shrimp and fish group) than for H3 (0.04 mg $larva^{-1}$ mm^{-1} for the shrimp group) and MT7 (0.02 mg $larva^{-1}$ mm^{-1} for both shrimp and fish groups). For both NL and TL, the slopes in groups H4-fish and H4-shrimp were significantly higher than group MT7-shrimp, MT7-fish (Figure D5; Tukey adjusted $P < 0.05$).

Saturated fatty acids (SFA) were higher in PC and PE than in TG (28.7 ± 2.0% in PC, 26.2 ± 5.1% in PE, 18.3 ± 2.8% in TG). Mono-unsaturated fatty acids (MUFA) were the highest in TG, followed by PC, and lowest in PE (23.8 ± 3.8% in TG, 20.6 ± 2.6% in PC, 13.2 ± 2.7% in PE). Poly-unsaturated fatty acid (PUFA) content was similar in all lipid classes (46.8 ± 7.4% in TG, 42.4 ± 3.0% in PC, 43.1 ± 5.1% in PE). Highly unsaturated fatty acids (HUFA) were higher in PC and PE than in TG (32.9 ± 6.0% for PC, 34.0 ± 4.9% for PE, 21.5 ± 8.8% for TG), with approximately twice as much n-3 HUFA than n-6 HUFA.

Table D5: Fatty acid profile (mean ± 1 S.D. of spawns, % total fatty acids) in triglyceride (TG) lipid class of red drum larvae from different broodstock tanks and maternal diets at 21 dph in Experiment 2.

Asterisks indicate fatty acids that differed significantly among maternal diet groups and/or females (two-way ANOVA, $P < 0.05$).

Fatty acid	H3		H4		MT7		Two-way ANOVA		
	Shrimp	Fish	Shrimp	Fish	Shrimp	Fish	Diet	Female	Interaction
14:0	1 ± 0.1	0.8 ± 0.2	1 ± 0.1	1 ± 0.1	0.8 ± 0.1	1 ± 0.1	-	-	-
15:0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	*	*	-
16:0	11 ± 1.3	10.8 ± 0.5	11.1 ± 1.1	10 ± 0.4	12.3 ± 0.9	12.6 ± 2.3	-	*	-
16:1n-7	2.7 ± 0.4	2.5 ± 0.9	2.4 ± 0.2	2 ± 0.1	3.1 ± 0.9	2.1 ± 0.3	*	-	-
16:2n-4	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0.1	0.2 ± 0.1	-	*	-
16:3n-4	0.8 ± 0.1	0.9 ± 0.3	0.7 ± 0.1	0.7 ± 0.1	1 ± 0.2	0.8 ± 0.2	-	*	-
17:0	0.7 ± 0.1	0.7 ± 0	0.6 ± 0.1	0.6 ± 0	0.8 ± 0.1	0.7 ± 0.1	-	*	-
18:0	4.7 ± 0.9	5.4 ± 0.2	4.4 ± 0.6	3.9 ± 0.5	6.3 ± 0.8	6.2 ± 1.8	-	*	-
18:1n-7	6.8 ± 0.8	7.2 ± 2.5	6 ± 0.6	5.2 ± 0.5	7.6 ± 1	6.2 ± 0.9	-	*	-
18:1n-9	14.6 ± 2.4	14.8 ± 2.2	13.5 ± 1.1	11.7 ± 1.1	16.2 ± 2.3	14.2 ± 1.6	-	*	-
18:2n-6	4.4 ± 0.2	4 ± 0.1	4.4 ± 0.2	4 ± 0.3	4.3 ± 0.3	4.1 ± 0.3	*	-	-
18:3n-3	18.2 ± 1.3	18.2 ± 3.3	17.2 ± 1.2	15.6 ± 1.6	17.6 ± 3.5	16.1 ± 1.9	-	-	-
18:3n-4	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.2 ± 0	-	-	-
18:3n-6	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	-	*	-
18:4n-3	2.4 ± 0.2	2.4 ± 0.4	2.2 ± 0.1	2 ± 0.2	2.2 ± 0.5	2.1 ± 0.2	-	-	-
20:1n-9	0.6 ± 0.1	0.8 ± 0.3	0.5 ± 0	0.4 ± 0.1	0.9 ± 0.4	0.6 ± 0.1	-	*	-

20:2n-6	0.2 ± 0	0.3 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.1	0.2 ± 0.1	-	-	-
20:3n-3	0.6 ± 0.1	0.8 ± 0	0.6 ± 0	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	-	*	-
20:3n-6	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0	0.3 ± 0	0.4 ± 0.2	0.4 ± 0.1	-	-	-
20:4n-3	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	-	-	-
20:4n-6	2.2 ± 0.7	2.1 ± 1.3	2.6 ± 0.4	3.4 ± 0.5	1.7 ± 0.6	2.5 ± 0.5	*	*	-
20:5n-3	3.2 ± 0.8	3.1 ± 1.6	3.7 ± 0.4	4.7 ± 0.4	2.8 ± 0.9	3.7 ± 0.8	*	*	-
22:1n-11	0.1 ± 0	0.1 ± 0.1	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0	-	-	-
22:4n-6	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0	0.4 ± 0	0.3 ± 0.1	0.3 ± 0.1	-	-	-
22:5n-3	0.3 ± 0.1	0.4 ± 0.3	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.4	0.4 ± 0.1	-	-	-
22:5n-6	3.5 ± 1.7	2.9 ± 3.4	4.9 ± 0.9	6.5 ± 1.2	1.7 ± 1.9	3.9 ± 1.6	*	*	-
22:6n-3	7.8 ± 4.2	6.1 ± 6.7	11.5 ± 2.3	14.8 ± 3.2	3.8 ± 3.7	8.1 ± 3.7	-	*	-

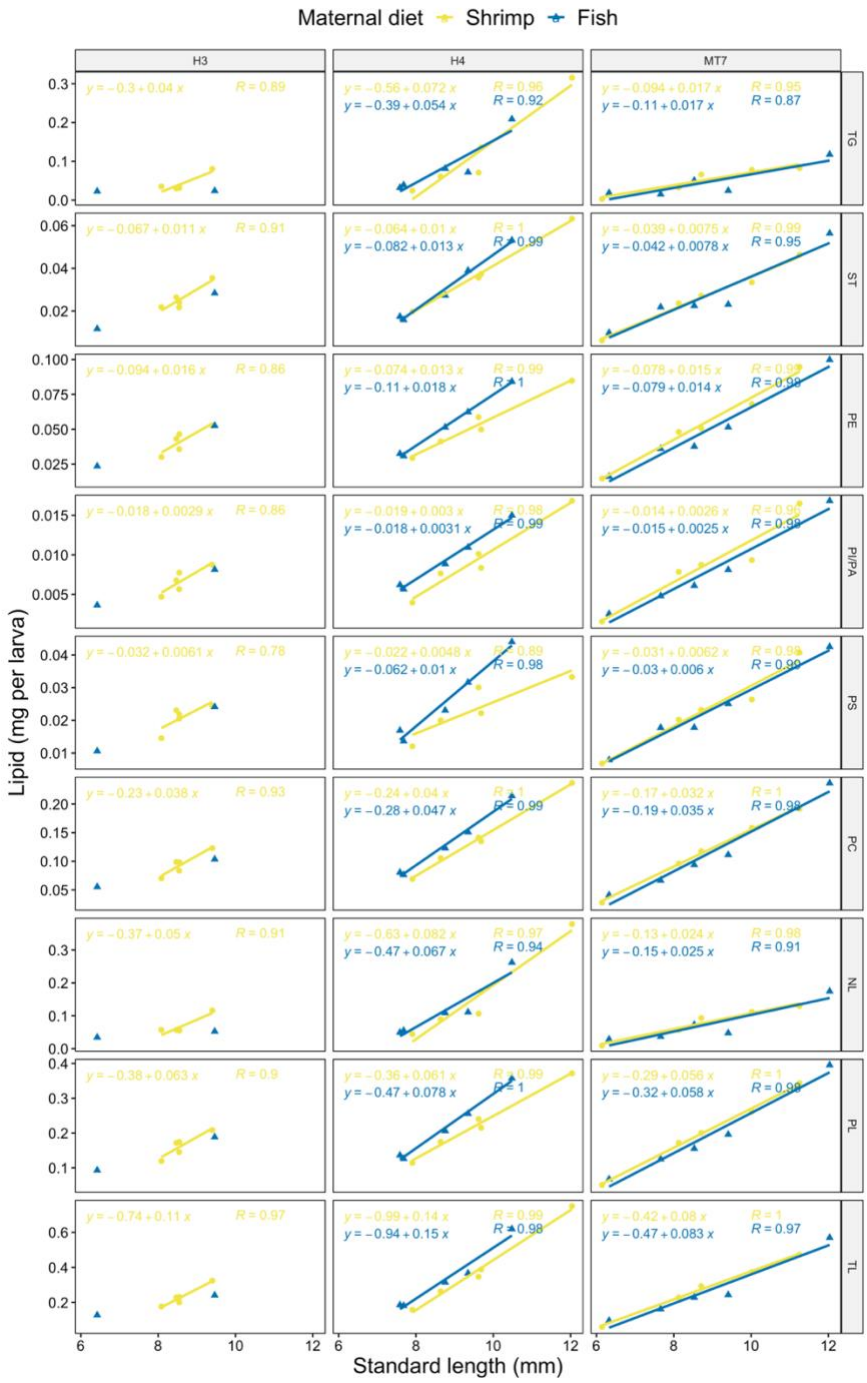

Figure D5: The relationship between the amount of lipids (mg larva^{-1}) and standard length (mm) of larvae in Experiment 2.

Rows represent lipid classes, or their group sums. Columns represent broodstock tanks. Colors indicate diet groups (dark blue: fish diet; yellow: shrimp diet). The equations and correlation coefficients (R) of the linear regression were shown in each cell.

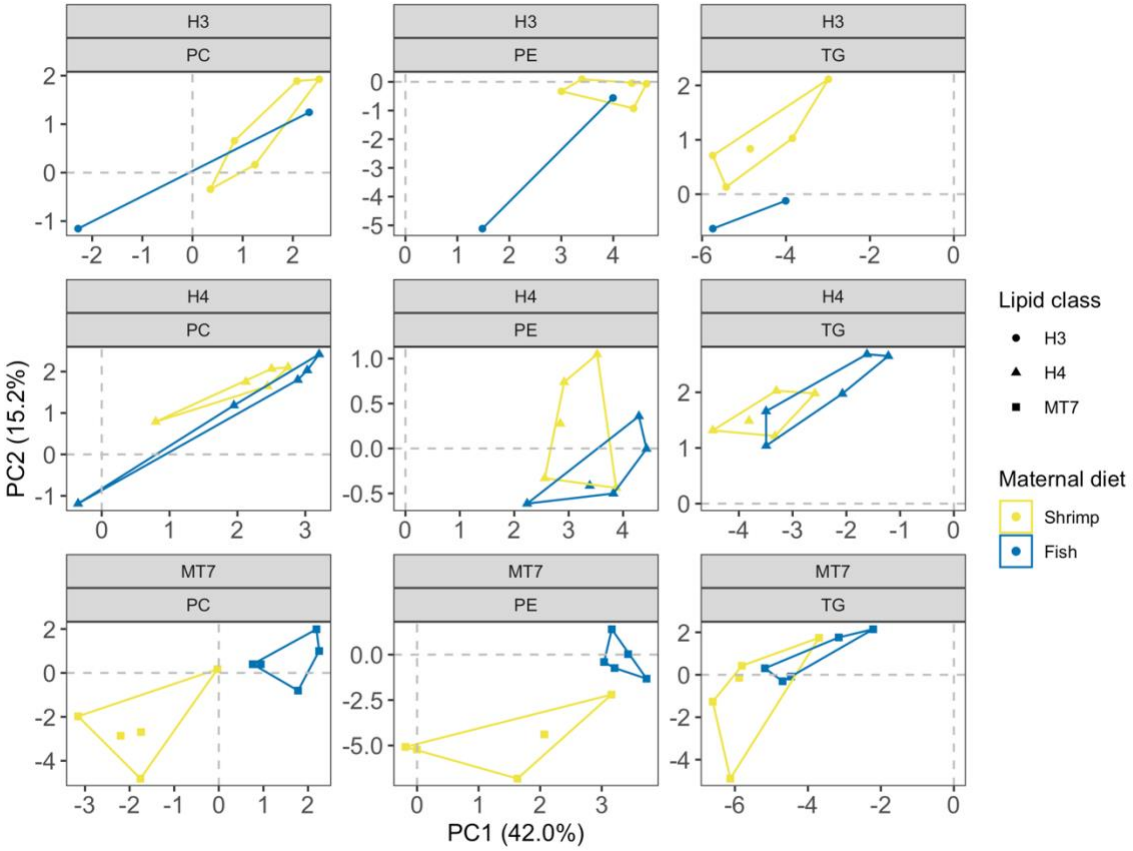

Figure D6: Principal component analysis of fatty acid composition (% total fatty acids) in lipid classes, PC, PE and TG, of red drum larvae produced by different adult fish that were fed different diets in Experiment 2.

Colors indicate diet groups (dark blue: fish diet; yellow: shrimp diet). Symbols indicate broodstock tanks (circle: H3; triangle: H4; square: MT7).

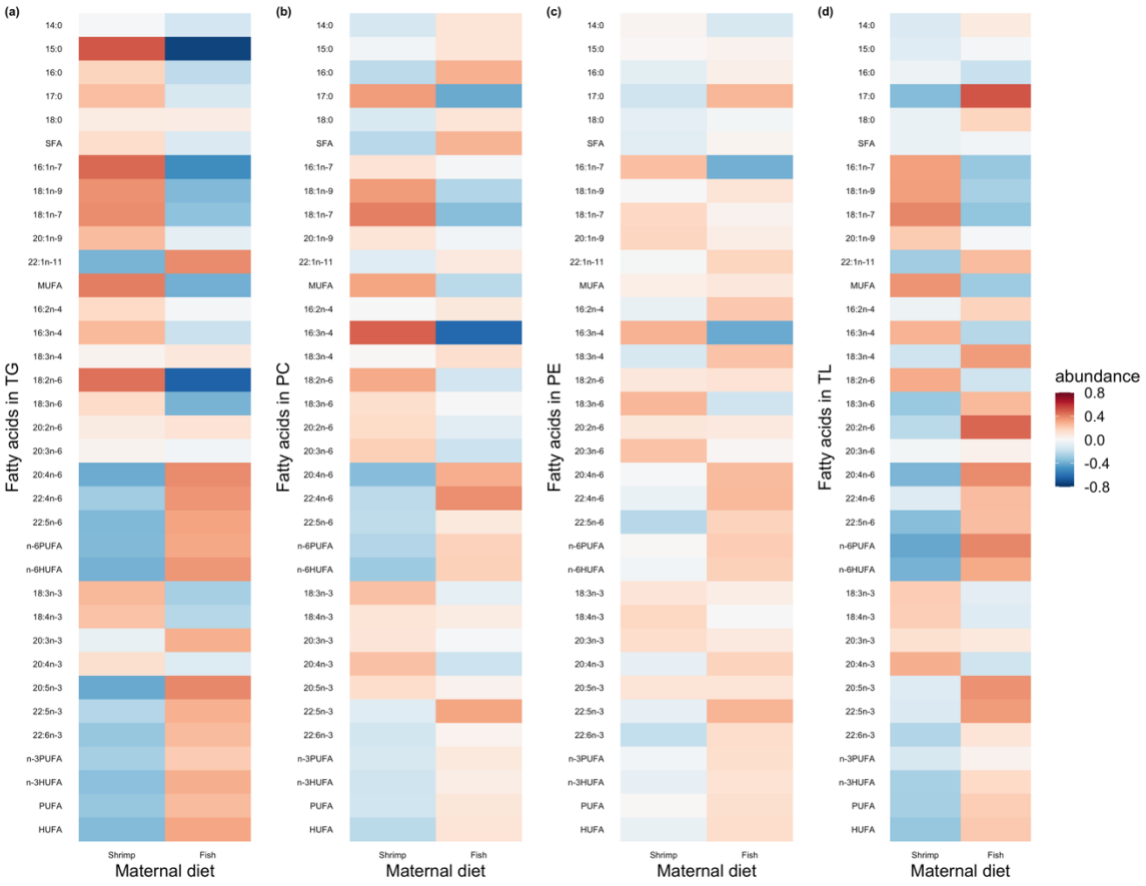

Figure D7: Heat map showing abundance of fatty acids and their sums (% total fatty acids) in (a) TG, (b) PC, (c) PE, (d) total lipids (TL) in Experiment 2.

Color of each cell indicates the mean abundance (n = 3 broodstock tanks) for each maternal diet group. Abundance data were scaled (mean = 0, sd = 1) separately for each broodstock tank. Columns represent diet groups from each broodstock tank. Rows represent individual fatty acids or their sums.

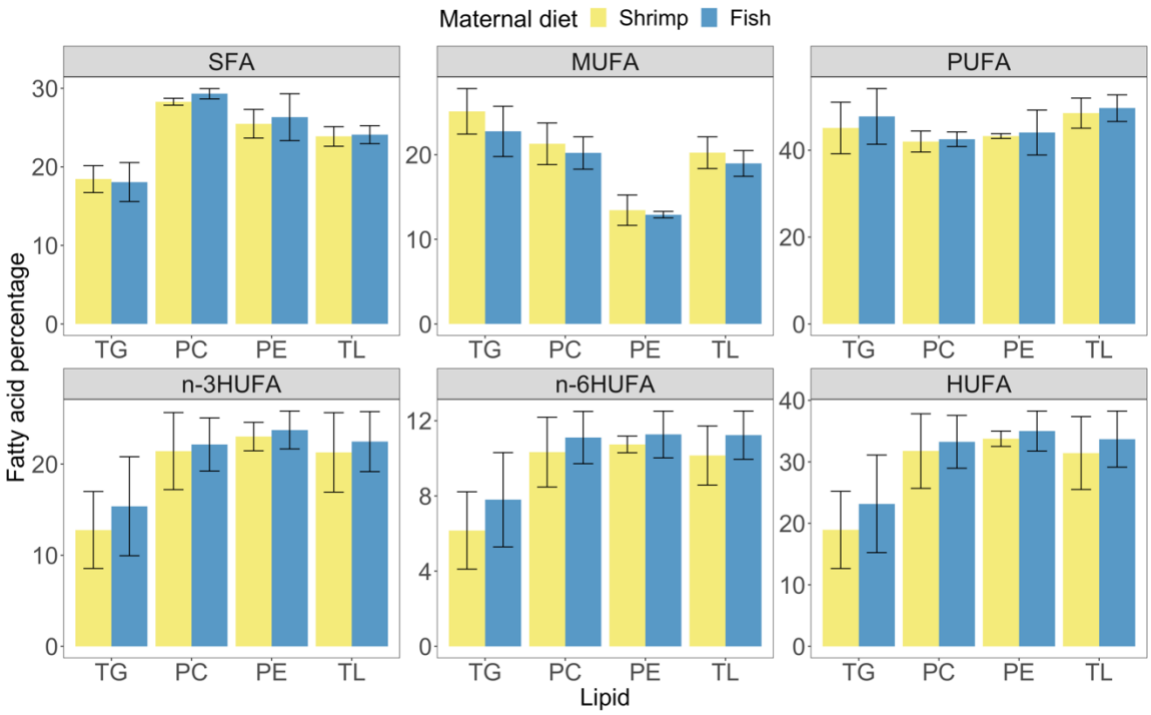

Figure D8: Percentages (mean ± 1 S.D.) of SFA, MUFA, PUFA, n-3 HUFA, n-6 HUFA, HUFA in TG, PC, PE and total lipids (TL) of larvae from different maternal diets at 21 dph.

References

Abreu S, Solgadi A, Chaminade P (2017) Optimization of normal phase chromatographic conditions for lipid analysis and comparison of associated detection techniques. J Chromatogr A 1514:54–71. doi: 10.1016/j.chroma.2017.07.063

Almansa E, Pérez MJ, Cejas JR, et al (1999) Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season. Aquaculture 170:323–336. doi: 10.1016/S0044-8486(98)00415-3

Anderson JT (1988) A review of size-dependent survival during pre-recruit stages of fishes in relation to recruitment. J Northwest Atl Fish Sci 55–66

Arnold CR, Bailey WH, Williams TD, et al (1977) Laboratory spawning and larval rearing of red drum and southern flounder. Proc Ann Conf Southeast Assoc Fish Wildl Agencies 31:437–440

Arzel J, Martinez Lopez FX, Métailler R, et al (1994) Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in seawater. Aquaculture 123:361–375. doi: 10.1016/0044-8486(94)90071-X

Athenstaedt K, Daum G (2006) The life cycle of neutral lipids: synthesis, storage and degradation. Cell Mol Life Sci CMLS 63:1355–1369. doi: 10.1007/s00018-006-6016-8

Bachan MM, Fleming IA, Trippel EA (2012) Maternal allocation of lipid classes and fatty acids with seasonal egg production in Atlantic cod (*Gadus morhua*) of wild origin. Mar Biol 159:2281–2297. doi: 10.1007/s00227-012-2014-6

Balasubramanian MN, Panserat S, Dupont-Nivet M, et al (2016) Molecular pathways associated with the nutritional programming of plant-based diet acceptance in rainbow trout following an early feeding exposure. BMC Genomics 17:1–20. doi: 10.1186/s12864-016-2804-1

Barnes H, Blackstock J (1973) Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanilun method for ‘total’ lipids. J Exp Mar Biol Ecol 12:103–118. doi: 10.1016/0022-0981(73)90040-3

Bell JG, Castell JD, Tocher DR, et al (1995) Effects of different dietary arachidonic acid : docosahexaenoic acid ratios on phospholipid fatty acid compositions and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). Fish Physiol Biochem 14:139–151. doi: 10.1007/BF00002457

Bell JG, Farndale BM, Bruce MP, et al (1997) Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture 149:107–119. doi: 10.1016/S0044-8486(96)01436-6

Bell JG, Henderson RJ, Tocher DR, et al (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. J Nutr 132:222–230

Bell JG, McEvoy J, Tocher DR, et al (2001) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and gepatocyte fatty acid metabolism. J Nutr 131:1535–1543

Bell JG, Sargent JR (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218:491–499. doi: 10.1016/S0044-8486(02)00370-8

Bell MV (1989) Molecular species analysis of phosphoglycerides from the ripe roes of cod (*Gadus morhua*). Lipids 24:585–588. doi: 10.1007/BF02535073

Bell MV, Dick JR, Thrush M, Navarro JC (1996) Decreased 20:4n-620:5n-3 ratio in sperm from cultured sea bass, *Dicentrarchus labrax*, broodstock compared with wild fish. Aquaculture 144:189–199. doi: 10.1016/S0044-8486(96)01311-7

Bell MV, Henderson RJ, Sargent JR (1986) The role of polyunsaturated fatty acids in fish. Comp Biochem Physiol Part B Comp Biochem 83:711–719. doi: 10.1016/0305-0491(86)90135-5

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57:289–300. doi: https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258–1264. doi: https://doi.org/10.1890/03-0706

Bonacic K, Campoverde C, Gómez-Arbonés J, et al (2016) Dietary fatty acid composition affects food intake and gut–brain satiety signaling in Senegalese sole (*Solea senegalensis*, Kaup 1858) larvae and post-larvae. Gen Comp Endocrinol 228:79–94. doi: 10.1016/j.ygcen.2016.02.002

Bransden MP, Battaglene SC, Goldsmid RM, et al (2007) Broodstock condition, egg morphology and lipid content and composition during the spawning season of captive striped trumpeter, *Latris lineata*. Aquaculture 268:2–12. doi: 10.1016/j.aquaculture.2007.04.026

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Mar Mammal Sci 22:759–801. doi: 10.1111/j.1748-7692.2006.00079.x

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29:1547–1559. doi: 10.1016/S0146-6380(98)00177-6

Budge SM, Parrish CC, Mckenzie CH (2001) Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. Mar Chem 76:285–303. doi: 10.1016/S0304-4203(01)00068-8

Budge SM, Penney SN, Lall SP (2012) Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. Can J Fish Aquat Sci 69:1033–1046. doi: 10.1139/f2012-039

Burns CM, Fuiman LA (2019) Maternally derived nutrients influence fatty acid composition and predator evasion behavior of larval southern flounder, *Paralichthys lethostigma*. J Exp Mar Biol Ecol 514–515:41–47. doi: 10.1016/j.jembe.2019.03.008

Burns CM, Fuiman LA (2020) Determining the position of southern flounder *Paralichthys lethostigma* on the reproductive energy allocation spectrum using an essential fatty acid as a maternal dietary biomarker. Environ Biol Fishes. doi: 10.1007/s10641-020-01013-3

Carter AB, Carton AG, McCormick MI, et al (2015) Maternal size, not age, influences egg quality of a wild, protogynous coral reef fish *Plectropomus leopardus*. Mar Ecol Prog Ser 529:249–263. doi: 10.3354/meps11277

Castro LFC, Tocher DR, Monroig O (2016) Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. Prog Lipid Res 62:25–40. doi: 10.1016/j.plipres.2016.01.001

Cejas JR, Almansa E, Jérez S, et al (2004) Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. Comp Biochem Physiol B Biochem Mol Biol 139:209–216. doi: 10.1016/j.cbpc.2004.07.010

Cejas JR, Almansa E, Villamandos JE, et al (2003) Lipid and fatty acid composition of ovaries from wild fish and ovaries and eggs from captive fish of white sea bream (*Diplodus sargus*). Aquaculture 216:299–313. doi: 10.1016/S0044-8486(02)00525-2

Chang G-Q, Gaysinskaya V, Karatayev O, Leibowitz SF (2008) Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. J Neurosci 28:12107–12119. doi: 10.1523/JNEUROSCI.2642-08.2008

Claustre H, C. Marty J, Cassiani L, Dagaut J (1989) Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea: Ecological implications. Mar Microb Food Webs 3:51–66

Copeman LA, Laurel BJ, Parrish CC (2013) Effect of temperature and tissue type on fatty acid signatures of two species of North Pacific juvenile gadids: A laboratory feeding study. J Exp Mar Biol Ecol 448:188–196. doi: 10.1016/j.jembe.2013.07.008

Couturier LIE, Michel LN, Amaro T, et al (2020) State of art and best practices for fatty acid analysis in aquatic sciences. ICES J Mar Sci. doi: 10.1093/icesjms/fsaa121

Crafts C, Plante M, Bailey B, Acworth IN (2016) Enhancement of linearity and response in charged aerosol detection. Thermo Fisher Scientific, Chelmsford, MA

Cripps RL, Martin-Gronert MS, Ozanne SE (2005) Fetal and perinatal programming of appetite. Clin Sci 109:1–11. doi: 10.1042/CS20040367

Cunha I, Galante-Oliveira S, Rocha E, et al (2013) Dynamics of PPARs, fatty acid metabolism genes and lipid classes in eggs and early larvae of a teleost. Comp Biochem Physiol B Biochem Mol Biol 164:247–258. doi: 10.1016/j.cbpb.2013.01.003

D'Alessandro M, Eugenia Oliva M, Alejandra Fortino M, Chicco A (2014) Maternal sucrose-rich diet and fetal programming: changes in hepatic lipogenic and oxidative enzymes and glucose homeostasis in adult offspring. Food Funct 5:446–453. doi: 10.1039/C3FO60436E

Dalsgaard J, St. John M (2004) Fatty acid biomarkers: validation of food web and trophic markers using 13C-labelled fatty acids in juvenile sandeel (*Ammodytes tobianus*). Can J Fish Aquat Sci 61:1671–1680. doi: 10.1139/f04-095

Dalsgaard J, St. John M, Kattner G, et al (2003a) Fatty acid trophic markers in the pelagic marine environment. In: Southward AJ, Tyler PA, Young CM, Fuiman LA (eds) Advances in Marine Biology. Academic Press, New York, NY, pp 225–340

Dalsgaard J, St. John M, Kattner G, et al (2003b) Fatty acid trophic markers in the pelagic marine environment. In: Biology B-A in M (ed). Academic Press, pp 225–340

Damsteegt EL, Mizuta H, Hiramatsu N, Lokman PM (2015) How do eggs get fat? Insights into ovarian fatty acid accumulation in the shortfinned eel, Anguilla australis. Gen Comp Endocrinol 221:94–100. doi: 10.1016/j.ygcen.2014.12.019

Derks R (2020) Rcpm: General functions for the Center of Proteomics and Metabolomics

Desvilettes C, Bourdier G, Breton JC (1997) Changes in lipid class and fatty acid composition during development in pike (*Esox lucius* L) eggs and larvae. Fish Physiol Biochem 16:381–393. doi: 10.1023/A:1007764130651

Dieterle F, Ross A, Schlotterbeck G, Senn H (2006) Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in ^{1}H NMR metabonomics. Anal Chem 78:4281–4290. doi: 10.1021/ac051632c

Duarte JAG, Carvalho F, Pearson M, et al (2014) A high-fat diet suppresses de novo lipogenesis and desaturation but not elongation and triglyceride synthesis in mice. J Lipid Res 55:2541–2553. doi: 10.1194/jlr.M052308

Dunstan GA, Volkman JK, Barrett SM, et al (1993) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35:155–161. doi: 10.1016/S0031-9422(00)90525-9

Eldridge MB, Joseph JD, Taberski KM, Seaborn GT (1983) Lipid and fatty acid composition of the endogenous energy sources of striped bass (*Morone saxatilis*) eggs. Lipids 18:510–513. doi: 10.1007/BF02535389

Engrola S, Aragão C, Valente LMP, Conceição LEC (2018) Nutritional modulation of marine fish larvae performance. In: Yúfera M (ed) Emerging issues in fish larvae research. Springer, Cham, pp 209–228

Eriksson J, Lindi V, Uusitupa M, et al (2003) The effects of the Pro12Ala polymorphism of the PPARγ-2 gene on lipid metabolism interact with body size at birth. Clin Genet 64:366–370. doi: https://doi.org/10.1034/j.1399-0004.2003.00150.x

Eriksson JG, Lindi V, Uusitupa M, et al (2002) The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 gene on insulin sensitivity and insulin metabolism interact with size at birth. Diabetes 51:2321–2324. doi: 10.2337/diabetes.51.7.2321

Faulk CK, Holt GJ (2005) Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: Live prey enrichment and greenwater culture. Aquaculture 249:231–243. doi: 10.1016/j.aquaculture.2005.03.033

Fernández-Palacios H, Norberg B, Izquierdo M, Hamre K (2011) Effects of broodstock diet on eggs and larvae. In: Holt GJ (ed) Larval Fish Nutrition. Wiley-Blackwell, John Wiley and Sons Publisher, Oxford, UK, pp 151–181

Ferramosca A, Zara V (2014) Modulation of hepatic steatosis by dietary fatty acids. World J Gastroenterol WJG 20:1746–1755. doi: 10.3748/wjg.v20.i7.1746

Finn RN, Fyhn HJ, Evjen MS (1995a) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. Mar Biol 124:355–369. doi: 10.1007/BF00363909

Finn RN, Fyhn HJ, Henderson RJ, Evjen MS (1996) The sequence of catabolic substrate oxidation and enthalpy balance of developing embryos and yolksac larvae of turbot (*Scophthalmus maximus* L.). Comp Biochem Physiol A Physiol 115:133–151. doi: 10.1016/0300-9629(96)00026-6

Finn RN, Henderson JR, Fyhn HJ (1995b) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). II. Lipid metabolism and enthalpy balance. Mar Biol 124:371–379. doi: 10.1007/BF00363910

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509

Fontagné-Dicharry S, Alami-Durante H, Aragão C, et al (2017) Parental and early-feeding effects of dietary methionine in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 469:16–27. doi: 10.1016/j.aquaculture.2016.11.039

Fraser AJ, Gamble JC, Sargent JR (1988) Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar Biol 99:307–313. doi: 10.1007/BF02112122

Fuiman LA (2002) Special consideration on fish eggs and larvae. In: Fuiman LA, Werner RG (eds) Fishery Science : The Unique Contributions of Early Life Stages. Blackwell Science, pp 1–32

Fuiman LA (2018) Egg boon fatty acids reveal effects of a climatic event on a marine food web. Ecol Monogr 88:585–599. doi: 10.1002/ecm.1324

Fuiman LA, Connelly TL, Lowerre-Barbieri SK, McClelland JW (2015a) Egg boons: central components of marine fatty acid food webs. Ecology 96:362–372. doi: 10.1890/14-0571.1

Fuiman LA, Connelly TL, Lowerre-Barbieri SK, McClelland JW (2015b) Egg boons: central components of marine fatty acid food webs. Ecology 96:362–372. doi: 10.1890/14-0571.1

Fuiman LA, Faulk CK (2013) Batch spawning facilitates transfer of an essential nutrient from diet to eggs in a marine fish. Biol Lett 9:20130593. doi: 10.1098/rsbl.2013.0593

Fuiman LA, Magurran AE (1994) Development of predator defences in fishes. Rev Fish Biol Fish 4:145–183. doi: 10.1007/BF00044127

Fuiman LA, Ojanguren AF (2011) Fatty acid content of eggs determines antipredator performance of fish larvae. J Exp Mar Biol Ecol 407:155–165. doi: 10.1016/j.jembe.2011.06.004

Fuiman LA, Perez KO (2015) Metabolic programming mediated by an essential fatty acid alters body composition and survival skills of a marine fish. Proc R Soc B Biol Sci 282:20151414. doi: 10.1098/rspb.2015.1414

Furuita H, Tanaka H, Yamamoto T, et al (2002) Effects of high levels of n−3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. Aquaculture 210:323–333. doi: 10.1016/S0044-8486(01)00855-9

Furuita H, Unuma T, Nomura K, et al (2006) Lipid and fatty acid composition of eggs producing larvae with high survival rate in the Japanese eel. J Fish Biol 69:1178–1189. doi: https://doi.org/10.1111/j.1095-8649.2006.01196.x

Galloway AWE, Budge SM (2020) The critical importance of experimentation in biomarker-based trophic ecology. Philos Trans R Soc B Biol Sci 375:20190638. doi: 10.1098/rstb.2019.0638

Garrido S, Rosa R, Ben-Hamadou R, et al (2007) Effect of maternal fat reserves on the fatty acid composition of sardine (*Sardina pilchardus*) oocytes. Comp Biochem Physiol B Biochem Mol Biol 148:398–409. doi: 10.1016/j.cbpb.2007.07.008

Garrido S, Rosa R, Ben-Hamadou R, et al (2008) Spatio-temporal variability in fatty acid trophic biomarkers in stomach contents and muscle of Iberian sardine (*Sardina pilchardus*) and its relationship with spawning. Mar Biol 154:1053–1065. doi: 10.1007/s00227-008-0999-7

Geurden I, Aramendi M, Zambonino-Infante J, Panserat S (2007) Early feeding of carnivorous rainbow trout (*Oncorhynchus mykiss*) with a hyperglucidic diet during a short period: effect on dietary glucose utilization in juveniles. Am J

Physiol - Regul Integr Comp Physiol 292:R2275–R2283. doi: 10.1152/ajpregu.00444.2006

Geurden I, Borchert P, Balasubramanian MN, et al (2013) The positive impact of the early-feeding of a plant-based diet on its future acceptance and utilisation in rainbow trout. PLOS ONE 8:e83162. doi: 10.1371/journal.pone.0083162

Geurden I, Mennigen J, Plagnes-Juan E, et al (2014) High or low dietary carbohydrate:protein ratios during first-feeding affect glucose metabolism and intestinal microbiota in juvenile rainbow trout. J Exp Biol 217:3396–3406. doi: 10.1242/jeb.106062

Giraldo C, Boutoute M, Mayzaud P, et al (2017) Lipid dynamics in early life stages of the icefish *Chionodraco hamatus* in the Dumont d'Urville Sea (East Antarctica). Polar Biol 40:313–320. doi: 10.1007/s00300-016-1956-4

Gong G, Xue M, Wang J, et al (2015) The regulation of gluconeogenesis in the Siberian sturgeon (*Acipenser baerii*) affected later in life by a short-term high-glucose programming during early life. Aquaculture 436:127–136. doi: 10.1016/j.aquaculture.2014.10.044

Green BS (2008) Maternal Effects in Fish Populations. In: Advances in Marine Biology. Elsevier, pp 1–105

Hadas E, Koven W, Sklan D, Tandler A (2003) The effect of dietary phosphatidylcholine on the assimilation and distribution of ingested free oleic acid (18:1n−9) in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 217:577–588. doi: 10.1016/S0044-8486(02)00431-3

Hamre K, Yúfera M, Rønnestad I, et al (2013) Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. Rev Aquac 5:S26–S58. doi: 10.1111/j.1753-5131.2012.01086.x

Happel A, Stratton L, Kolb C, et al (2016) Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. Can J Fish Aquat Sci 73:1222–1229. doi: 10.1139/cjfas-2015-0328

Harayama T, Shimizu T (2020) Roles of polyunsaturated fatty acids, from mediators to membranes. J Lipid Res jlr.R120000800. doi: 10.1194/jlr.R120000800

Hardy RW, Scott TM, Harrell LW (1987) Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. Aquaculture 65:267–277. doi: 10.1016/0044-8486(87)90240-7

Harel M, Tandler A, Kissil GW, Applebaum SW (1994) The kinetics of nutrient incorporation into body tissues of gilthead seabream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality. Br J Nutr 72:45–58. doi: 10.1079/BJN19940008

He A-Y, Ning L-J, Chen L-Q, et al (2015) Systemic adaptation of lipid metabolism in response to low- and high-fat diet in Nile tilapia (*Oreochromis niloticus*). Physiol Rep 3:e12485. doi: https://doi.org/10.14814/phy2.12485

Heming TA, Buddington RK (1988) Yolk absorption in embryonic and larval fishes. In: Hoar WS, Randall DJ (eds) Fish Physiology. Academic Press, San Diego, CA, pp 407–446

Henderson JR, Tocher DR (1987) The lipid composition and biochemistry of freshwater fish. Prog Lipid Res 26:281–347. doi: 10.1016/0163-7827(87)90002-6

Henderson RJ, Sargent JR, Hopkins CCE (1984) Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. Mar Biol 78:255–263. doi: 10.1007/BF00393011

Hiramatsu N, Luo W, Reading BJ, et al (2012) Multiple ovarian lipoprotein receptors in teleosts. Fish Physiol Biochem 39:29–32. doi: 10.1007/s10695-012-9612-6

Holt J, Johnson AG, Arnold CR, et al (1981) Description of Eggs and Larvae of Laboratory Reared Red Drum, Sciaenops ocellata. Copeia 1981:751–756. doi: 10.2307/1444174

Hothorn T, Hornik K (2021) exactRankTests: Exact Distributions for Rank and Permutation Tests. Comprehensive R Archive Network (CRAN)

Hou Z, Faulk CK, Fuiman LA (2020) Dynamics of diet-egg transfer of fatty acids in the teleost fish, red drum (*Sciaenops ocellatus*). Philos Trans R Soc B Biol Sci 375:20190646. doi: 10.1098/rstb.2019.0646

Hou Z, Fuiman LA (2021) Maternal diet affects utilization of endogenous lipids by red drum *Sciaenops ocellatus* embryos and early larvae. Comp Biochem Physiol B Biochem Mol Biol 256:110639. doi: 10.1016/j.cbpb.2021.110639

Hou Z, Fuiman LA (2020) Nutritional programming in fishes: insights from mammalian studies. Rev Fish Biol Fish 30:67–92. doi: 10.1007/s11160-019-09590-y

Houde ED (2002) Mortality. In: Fuiman LA, Werner RG (eds) Fishery Science : The Unique Contributions of Early Life Stages. Blackwell, Oxford, pp 64–87

Houde ED (1997) Patterns and trends in larval-stage growth and mortality of teleost fish. J Fish Biol 51:52–83. doi: 10.1111/j.1095-8649.1997.tb06093.x

Huynh MD, Kitts DD, Hu C, Trites AW (2007) Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. Comp Biochem Physiol B Biochem Mol Biol 146:504–511. doi: 10.1016/j.cbpb.2006.11.023

Iritani N, Yamashita S, Numa S (1976) Dietary control of triglyceride and phospholipid synthesis in rat liver slices. J Biochem (Tokyo) 80:217–222

Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Kainz M, Brett MT, Arts MT (eds) Lipids in Aquatic Ecosystems. Springer New York, New York, NY, pp 281–308

Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecol Monogr 74:211–235. doi: 10.1890/02-4105

Izquierdo MS (1996) Essential fatty acid requirements of cultured marine fish larvae. Aquac Nutr 2:183–191. doi: 10.1111/j.1365-2095.1996.tb00058.x

Izquierdo MS, Fernández-Palacios H, Tacon AGJ (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25–42. doi: 10.1016/S0044-8486(01)00581-6

Izquierdo MS, Socorro J, Arantzamendi L, Hernández-Cruz CM (2000) Recent advances in lipid nutrition in fish larvae. Fish Physiol Biochem 22:97–107. doi: 10.1023/A:1007810506259

Izquierdo MS, Turkmen S, Montero D, et al (2015) Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. Aquaculture 449:18–26. doi: 10.1016/j.aquaculture.2015.03.032

Jaroszewska M, Dabrowski K (2011) Utilization of yolk: transition from endogenous to exogenous nutrition in fish. In: Holt GJ (ed) Larval Fish Nutrition. John Wiley & Sons, Ltd, pp 183–218

Jobling M (2004) Are modifications in tissue fatty acid profiles following a change in diet the result of dilution?: Test of a simple dilution model. Aquaculture 232:551–562. doi: 10.1016/j.aquaculture.2003.07.001

Johnson RB (2009) Lipid deposition in oocytes of teleost fish during secondary oocyte growth. Rev Fish Sci 17:78–89. doi: 10.1080/10641260802590004

Johnson RB, Kroeger EL, Carter CS, et al (2011) Transitioning coho salmon broodstock to a docosahexaenoic acid (DHA)-rich diet during vitellogenesis: effects on egg composition and embryo and fry quality. North Am J Aquac 73:409–417. doi: 10.1080/15222055.2011.623942

Johnson RB, Kroeger EL, Reichert WL, et al (2017) Uptake and selective partitioning of dietary lipids to ovarian and muscle tissue of maturing female coho salmon, *Oncorhynchus kisutch*, during secondary oocyte growth. Comp Biochem Physiol B Biochem Mol Biol 208–209:7–18. doi: 10.1016/j.cbpb.2017.03.005

Jönsson KI (1997) Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78:57–66. doi: 10.2307/3545800

Kabeya N, Yamamoto Y, Cummins SF, et al (2015) Polyunsaturated fatty acid metabolism in a marine teleost, Nibe croaker *Nibea mitsukurii*: Functional characterization of Fads2 desaturase and Elovl5 and Elovl4 elongases. Comp Biochem Physiol B Biochem Mol Biol 188:37–45. doi: 10.1016/j.cbpb.2015.06.005

Kaitaranta JK, Ackman RG (1981) Total lipids and lipid classes of fish roe. Comp Biochem Physiol Part B Comp Biochem 69:725–729. doi: 10.1016/0305-0491(81)90376-X

Kamler E (2008) Resource allocation in yolk-feeding fish. Rev Fish Biol Fish 18:143–200. doi: 10.1007/s11160-007-9070-x

Kassambara A (2020) rstatix: Pipe-Friendly Framework for Basic Statistical Tests. Comprehensive R Archive Network (CRAN)

Kolodzey S, Wing S, Durante L (2021) Maternal age, length, and weight influence larval characteristics and viability in the viviparous temperate reef fish *Helicolenus percoides*. Mar Ecol Prog Ser 659:185–198. doi: 10.3354/meps13542

Lane RH, Kelley DE, Ritov VH, et al (2001) Altered expression and function of mitochondrial β-oxidation enzymes in juvenile intrauterine-growth-retarded rat skeletal muscle. Pediatr Res 50:83–90. doi: 10.1203/00006450-200107000-00016

Lane RL, Kohler CC (2006) Effects of dietary lipid and fatty acids on white bass reproductive performance, egg hatchability, and overall quality of progeny. North Am J Aquac 68:141–150. doi: 10.1577/A05-009.1

Lauritzen L, Brambilla P, Mazzocchi A, et al (2016) DHA Effects in Brain Development and Function. Nutrients 8:6. doi: 10.3390/nu8010006

Lazo JP, Darias MJ, Gisbert E (2011) Ontogeny of the digestive tract. In: Holt GJ (ed) Larval Fish Nutrition. Wiley-Blackwell, John Wiley and Sons Publisher, Oxford, UK, pp 5–46

Leaver MJ, Taggart JB, Villeneuve L, et al (2011) Heritability and mechanisms of n−3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comp Biochem Physiol Part D Genomics Proteomics 6:62–69. doi: 10.1016/j.cbd.2010.04.002

Leslie CC (2004) Regulation of arachidonic acid availability for eicosanoid production. Biochem Cell Biol 82:1–17. doi: 10.1139/o03-080

Lewis HA, Trushenski JT, Lane RL, Kohler CC (2011) Differential incorporation of dietary fatty acids from flax and fish oils into lipid classes of white bass ova. North Am J Aquac 73:212–220. doi: https://doi.org/10.1080/15222055.2011.579030

Li D, Weisinger HS, Weisinger RS, et al (2006) Omega 6 to omega 3 fatty acid imbalance early in life leads to persistent reductions in DHA levels in glycerophospholipids in rat hypothalamus even after long-term omega 3 fatty acid repletion. Prostaglandins Leukot Essent Fatty Acids 74:391–399. doi: 10.1016/j.plefa.2006.03.010

Li Y, Chen W, Sun Z, et al (2005) Effects of n-3 HUFA content in broodstock diet on spawning performance and fatty acid composition of eggs and larvae in *Plectorhynchus cinctus*. Aquaculture 245:263–272. doi: 10.1016/j.aquaculture.2004.12.016

Litvin SY, Guida VG, Weinstein MP (2011) Lipid class dynamics and storage depots in juvenile weakfish *Cynoscion regalis* and their application to condition assessment. J Fish Biol 78:1294–1310. doi: https://doi.org/10.1111/j.1095-8649.2011.02918.x

Lu K-L, Xu W-N, Li X-F, et al (2013) Hepatic triacylglycerol secretion, lipid transport and tissue lipid uptake in blunt snout bream (*Megalobrama amblycephala*) fed high-fat diet. Aquaculture 408–409:160–168. doi: 10.1016/j.aquaculture.2013.06.003

Lu X, Lodi A, Konopleva M, Tiziani S (2019) Three-dimensional leukemia co-culture system for in vitro high-content metabolomics screening. SLAS Discov Adv Sci Drug Discov 24:817–828. doi: 10.1177/2472555219860446

Luan J, Browne PO, Harding A-H, et al (2001) Evidence for gene-nutrient interaction at the PPARγ locus. Diabetes 50:686–689. doi: 10.2337/diabetes.50.3.686

Lubzens E, Young G, Bobe J, Cerdà J (2010) Oogenesis in teleosts: How fish eggs are formed. Gen Comp Endocrinol 165:367–389. doi: 10.1016/j.ygcen.2009.05.022

Lucas A (1991) Programming by early nutrition in man. In: Bock GR, Whelan J (eds) The childhood environment and adult disease. CIBA Foundation Symposium 156. Wiley, Chichester, UK, pp 38–55

Lund I, Skov PV, Hansen BW (2012) Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. Comp Biochem Physiol A Mol Integr Physiol 162:340–348. doi: 10.1016/j.cbpa.2012.04.004

Madsen L, Frøyland L, Dyrøy E, et al (1998) Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. J Lipid Res 39:583–593

Martins DA, Rocha F, Martínez-Rodríguez G, et al (2012) Teleost fish larvae adapt to dietary arachidonic acid supply through modulation of the expression of lipid metabolism and stress response genes. Br J Nutr 108:864–874. doi: 10.1017/S0007114511006143

McBride RS, Somarakis S, Fitzhugh GR, et al (2015) Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish Fish 16:23–57. doi: 10.1111/faf.12043

Mejri S, Burns CM, Hou Z, Fuiman LA (2021) Maternal allocation of lipids to eggs by southern flounder after diet changes. North Am J Aquac 83:41–48. doi: https://doi.org/10.1002/naaq.10175

Mejri S, Luck C, Tremblay R, et al (2018) Bonefish (*Albula vulpes*) oocyte lipid class and fatty acid composition related to their development. Environ Biol Fishes. doi: 10.1007/s10641-018-0825-0

Miller TJ, Crowder LB, Rice JA, Marschall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can J Fish Aquat Sci 45:1657–1670. doi: 10.1139/f88-197

Mohan SD, Mohan JA, Connelly TL, et al (2016) Fatty-acid biomarkers and tissue-specific turnover: validation from a controlled feeding study in juvenile Atlantic croaker *Micropogonias undulatus*. J Fish Biol 89:2004–2023. doi: 10.1111/jfb.13099

Monroig O, Tocher DR, Castro LFC (2018) Polyunsaturated fatty acid biosynthesis and metabolism in fish. In: Burdge GC (ed) Polyunsaturated Fatty Acid Metabolism. Academic Press and AOCS Press, London, pp 31–60

Morais S, Castanheira F, Martinez-Rubio L, et al (2012a) Long chain polyunsaturated fatty acid synthesis in a marine vertebrate: Ontogenetic and nutritional regulation of a fatty acyl desaturase with Δ4 activity. Biochim Biophys Acta BBA - Mol Cell Biol Lipids 1821:660–671. doi: 10.1016/j.bbalip.2011.12.011

Morais S, Koven W, Rønnestad I, et al (2005) Dietary protein:lipid ratio and lipid nature affects fatty acid absorption and metabolism in a teleost larva. Br J Nutr 93:813–820. doi: 10.1079/BJN20051378

Morais S, Mendes AC, Castanheira MF, et al (2014) New formulated diets for *Solea senegalensis* broodstock: Effects of parental nutrition on biosynthesis of long-chain polyunsaturated fatty acids and performance of early larval stages and juvenile fish. Aquaculture 432:374–382. doi: 10.1016/j.aquaculture.2014.04.033

Morais S, Pratoomyot J, Taggart JB, et al (2011a) Genotype-specific responses in Atlantic salmon (*Salmo salar*) subject to dietary fish oil replacement by vegetable oil: a liver transcriptomic analysis. BMC Genomics 12:255. doi: 10.1186/1471-2164-12-255

Morais S, Pratoomyot J, Torstensen BE, et al (2011b) Diet × genotype interactions in hepatic cholesterol and lipoprotein metabolism in Atlantic salmon (*Salmo salar*) in response to replacement of dietary fish oil with vegetable oil. Br J Nutr 106:1457–1469. doi: 10.1017/S0007114511001954

Morais S, Taggart JB, Guy DR, et al (2012b) Hepatic transcriptome analysis of inter-family variability in flesh n-3 long-chain polyunsaturated fatty acid content in Atlantic salmon. BMC Genomics 13:410. doi: 10.1186/1471-2164-13-410

Mourente G, Megina C, Dıaz-Salvago E (2002) Lipids in female northern bluefin tuna (*Thunnus thynnus thynnus* L.) during sexual maturation. Fish Physiol Biochem 13

Mourente G, Odriozola JM (1990) Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). Fish Physiol Biochem 8:93–101. doi: 10.1007/BF00004436

Mourente G, Rodríguez A, Grau A, Pastor E (1999) Utilization of lipids by *Dentex dentex* L. (Osteichthyes, Sparidae) larvae during lecitotrophia and subsequent starvation. Fish Physiol Biochem 21:45–58. doi: 10.1023/A:1007789908409

Mourente G, Vázquez R (1996) Changes in the content of total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. Fish Physiol Biochem 15:221–235. doi: 10.1007/BF01875573

Murzina SA, Meyer Ottesen CA, Falk-Petersen S, et al (2012) Oogenesis and lipids in gonad and liver of daubed shanny (*Leptoclinus maculatus*) females from Svalbard waters. Fish Physiol Biochem 38:1393–1407. doi: 10.1007/s10695-012-9627-z

Mustafa T, Srivastava KC (1989) Prostaglandins (eicosanoids) and their role in ectothermic organisms. In: Brouwer M, Carr WES, Ellington WR, et al. (eds) Advances in Comparative and Environmental Physiology. Springer, Berlin, Heidelberg, pp 157–207

Nakamura MT, Nara TY (2003) Essential fatty acid synthesis and its regulation in mammals. Prostaglandins Leukot Essent Fatty Acids 68:145–150. doi: 10.1016/S0952-3278(02)00264-8

Nakanon N, Shimizu S, Yoshizumi H (1999) Formation of 8, 11, 14-octadecatrienoic acid (18:3 n-4) from naturally occurring unique fatty acid, 9, 12-hexadecadienoic acid (16:2 n-4), in animal cell cultures. J Nutr Sci Vitaminol 45:10

Navas JM, Bruce M, Thrush M, et al (1997) The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. J Fish Biol 51:760–773. doi: 10.1111/j.1095-8649.1997.tb01997.x

Norambuena F, Lewis M, Hamid NKA, et al (2013) Fish oil replacement in current aquaculture feed: Is cholesterol a hidden treasure for fish nutrition? PLOS ONE 8:e81705. doi: 10.1371/journal.pone.0081705

Norton EC, MacFarlane RB, Mohr MS (2001) Lipid class dynamics during development in early life stages of shortbelly rockfish and their application to condition assessment. J Fish Biol 58:1010–1024. doi: https://doi.org/10.1111/j.1095-8649.2001.tb00551.x

Oberg EW, Fuiman LA (2015) Linking fatty acids in the diet and tissues to quality of larval southern flounder (*Paralichthys lethostigma*). J Exp Mar Biol Ecol 467:7–15. doi: 10.1016/j.jembe.2015.02.021

Olsen R, Ringø E (1997) Lipid digestibility in fish: a review. In: Recent Res. Dev. Lipid Res. pp 199–265

Ozanne SE (2015) Metabolic programming—knowns, unknowns and possibilities. Nat Rev Endocrinol 11:67–68. doi: 10.1038/nrendo.2014.218

Panserat S, Marandel L, Geurden I, et al (2017) Muscle catabolic capacities and global hepatic epigenome are modified in juvenile rainbow trout fed different vitamin levels at first feeding. Aquaculture 468:515–523. doi: 10.1016/j.aquaculture.2016.11.021

Panserat S, Marandel L, Seiliez I, Skiba-Cassy S (2019) New insights on intermediary metabolism for a better understanding of nutrition in teleosts. Annu Rev Anim Biosci 7:195–220. doi: 10.1146/annurev-animal-020518-115250

Parish EJ, Boos TL, Li SR (2008) The chemistry of waxes and sterols. In: Akoh CC, Min DB (eds) Food lipids: chemistry, nutrition, and biotechnology. CRC Press, Boca Raton, FL., USA, pp 99–123

Patsouris D, Reddy JK, Müller M, Kersten S (2006) Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression. Endocrinology 147:1508–1516. doi: 10.1210/en.2005-1132

Perera E, Yúfera M (2016a) Soybean meal and soy protein concentrate in early diet elicit different nutritional programming effects on juvenile zebrafish. Zebrafish 13:61–69. doi: 10.1089/zeb.2015.1131

Perera E, Yúfera M (2016b) Effects of soybean meal on digestive enzymes activity, expression of inflammation-related genes, and chromatin modifications in marine fish (*Sparus aurata* L.) larvae. Fish Physiol Biochem 1–16. doi: 10.1007/s10695-016-0310-7

Perez KO, Fuiman LA (2015) Maternal diet and larval diet influence survival skills of larval red drum *Sciaenops ocellatus*. J Fish Biol 86:1286–1304. doi: 10.1111/jfb.12637

Phan CT, Tso P (2001) Intestinal lipid absorption and transport. Front Biosci J Virtual Libr 6:D299-319

Pittman K, Yúfera M, Pavlidis M, et al (2013) Fantastically plastic: fish larvae equipped for a new world. Rev Aquac 5:S224–S267. doi: 10.1111/raq.12034

Plagemann A, Harder T, Rake A, et al (2000) Hypothalamic nuclei are malformed in weanling offspring of low protein malnourished rat dams. J Nutr 130:2582–2589. doi: 10.1093/jn/130.10.2582

Plante M, Bailey B, Acworth IN (2016) Analysis of phospholipids in natural samples by normal phase HPLC and Corona charged aerosol detection. Thermo Fisher Scientific, Chelmsford, MA

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rainuzzo JR, Reitan KI, Olsen Y (1997) The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103–115. doi: 10.1016/S0044-8486(97)00121-X

Reading BJ, Sullivan CV, Schilling J (2017) Vitellogenesis in fishes. In: Reference Module in Life Sciences. Elsevier

Reddy JK, Mannaerts GP (1994) Peroxisomal lipid metabolism. Annu Rev Nutr 14:343–370. doi: 10.1146/annurev.nu.14.070194.002015

Rinchard J, Kestemont P (1996) Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and histological features. J Fish Biol 49:883–894. doi: https://doi.org/10.1111/j.1095-8649.1996.tb00087.x

Rinchard J, Kestemont P (2003) Liver changes related to oocyte growth in roach, a single spawner fish, and in bleak and white bream, two multiple spawner fish. Int Rev Hydrobiol 88:68–76. doi: https://doi.org/10.1002/iroh.200390006

Robin JH, Regost C, Arzel J, Kaushik SJ (2003) Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. Aquaculture 225:283–293. doi: 10.1016/S0044-8486(03)00296-5

Rocha F, Dias J, Geurden I, et al (2016a) High-glucose feeding of gilthead seabream (*Sparus aurata*) larvae: Effects on molecular and metabolic pathways. Aquaculture 451:241–253. doi: 10.1016/j.aquaculture.2015.09.015

Rocha F, Dias J, Geurden I, et al (2016b) Dietary glucose stimulus at larval stage modifies the carbohydrate metabolic pathway in gilthead seabream (*Sparus aurata*) juveniles: An in vivo approach using ^{14}C-starch. Comp Biochem Physiol A Mol Integr Physiol 201:189–199. doi: 10.1016/j.cbpa.2016.07.016

Rønnestad I, Finn RN, Groot EP, Fyhn HJ (1992) Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole *Microstomus kitt* reared in the laboratory. Mar Ecol Prog Ser 88:195–205

Rønnestad I, Finn RN, Lie Ø, Lein I (1995) Compartmental changes in the contents of total lipid, lipid classes and their associated fatty acids in developing yolk-sac larvae of Atlantic halibut, *Hippoglossus hippoglossus* (L.). Aquac Nutr 1:119–130. doi: 10.1111/j.1365-2095.1995.tb00027.x

Rønnestad I, Koven W, Tandler A, et al (1998) Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). Aquaculture 162:157–170. doi: 10.1016/S0044-8486(98)00203-8

Rønnestad I, Koven WM, Tandler A, et al (1994) Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). Mar Biol 120:187–196. doi: 10.1007/BF00349678

Rønnestad I, Yúfera M, Ueberschär B, et al (2013) Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Rev Aquac 5:S59–S98. doi: 10.1111/raq.12010

Salhi M, Hernández-Cruz CM, Bessonart M, et al (1999) Effect of different dietary polar lipid levels and different n−3 HUFA content in polar lipids on gut and liver histological structure of gilthead seabream (*Sparus aurata*) larvae. Aquaculture 179:253–263. doi: 10.1016/S0044-8486(99)00194-5

Salze G, Tocher DR, Roy WJ, Robertson DA (2005) Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. Aquac Res 36:1488–1499. doi: 10.1111/j.1365-2109.2005.01367.x

Sand DM, Hehl JL, Schlenk H (1969) Biosynthesis of wax esters in fish. Reduction of fatty acids and oxidation of alcohols. Biochemistry 8:4851–4854. doi: 10.1021/bi00840a030

Sargent J, Bell G, McEvoy L, et al (1999a) Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191–199. doi: 10.1016/S0044-8486(99)00083-6

Sargent J, McEvoy L, Estevez A, et al (1999b) Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229. doi: 10.1016/S0044-8486(99)00191-X

Sargent J r., Bell J g., Bell M v., et al (1993) The metabolism of phospholipids and polyunsaturated fatty acids in fish. In: Lahlou B, Vitiello P (eds) Aquaculture: Fundamental and Applied Research. American Geophysical Union, Washington, D.C., pp 103–124

Sargent J, Tocher D, Bell J (2002) The lipids. In: Halver JE, Hardy RW (eds) Fish nutrition, Third edition. Academic Press, San Diego, California, pp 181–257

Sargent JR, McEvoy LA, Bell JG (1997) Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155:117–127. doi: 10.1016/S0044-8486(97)00122-1

Seiliez I, Vélez EJ, Lutfi E, et al (2017) Eating for two: Consequences of parental methionine nutrition on offspring metabolism in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 471:80–91. doi: 10.1016/j.aquaculture.2017.01.010

Sheridan MA (1988) Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp Biochem Physiol Part B Comp Biochem 90:679–690. doi: 10.1016/0305-0491(88)90322-7

Silversand C (1996) Vitellogenesis in teleost fish. A study of vitellogenin and egg lipids. Doctoral thesis, University of Gothenburg

Silversand C, Haux C (1995) Fatty acid composition of vitellogenin from four teleost species. J Comp Physiol B 164:593–599. doi: 10.1007/BF00389799

Silversand C, Haux C (1997) Improved high-performance liquid chromatographic method for the separation and quantification of lipid classes: application to fish lipids. J Chromatogr B Biomed Sci App 703:7–14. doi: 10.1016/S0378-4347(97)00385-X

Silversand C, Norberg B, Haux C (1996) Fatty-acid composition of ovulated eggs from wild and cultured turbot (*Scophthalmus maximus*) in relation to yolk and oil globule lipids. Mar Biol 125:269–278. doi: 10.1007/BF00346307

Skjærven KH, Jakt LM, Fernandes JMO, et al (2018) Parental micronutrient deficiency distorts liver DNA methylation and expression of lipid genes associated with a fatty-liver-like phenotype in offspring. Sci Rep 8:1–16. doi: 10.1038/s41598-018-21211-5

Small DM (1991) The effects of glyceride structure on absorption and metabolism. Annu Rev Nutr 11:413–434. doi: 10.1146/annurev.nu.11.070191.002213

St. John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131:75–85. doi: 10.3354/meps131075

Symonds ME, Sebert SP, Hyatt MA, Budge H (2009) Nutritional programming of the metabolic syndrome. Nat Rev Endocrinol 5:604–610. doi: 10.1038/nrendo.2009.195

Tanaka T, Iwawaki D, Sakamoto M, et al (2003) Mechanisms of accumulation of arachidonate in phosphatidylinositol in yellowtail. Eur J Biochem 270:1466–1473. doi: 10.1046/j.1432-1033.2003.03512.x

Tang C, Cho HP, Nakamura MT, Clarke SD (2003) Regulation of human Δ-6 desaturase gene transcription identification of a functional direct repeat-1 element. J Lipid Res 44:686–695. doi: 10.1194/jlr.M200195-JLR200

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107–184. doi: 10.1080/713610925

Tocher DR (2015) Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449:94–107. doi: 10.1016/j.aquaculture.2015.01.010

Tocher DR, Bendiksen EÅ, Campbell PJ, Bell JG (2008) The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280:21–34. doi: 10.1016/j.aquaculture.2008.04.034

Tocher DR, Glencross BD (2015) Lipids and fatty acids. In: Dietary Nutrients, Additives, and Fish Health. John Wiley & Sons, Ltd, pp 47–94

Tocher DR, Sargent JR (1984) Analyses of lipids and fatty acids in ripe roes of some Northwest European marine fish. Lipids 19:492–499. doi: 10.1007/BF02534481

Trujillo E, Davis C, Milner J (2006) Nutrigenomics, proteomics, metabolomics, and the practice of dietetics. J Am Diet Assoc 106:403–413. doi: 10.1016/j.jada.2005.12.002

Turkmen S, Hernández-Cruz CM, Zamorano MJ, et al (2019a) Long-chain PUFA profiles in parental diets induce long-term effects on growth, fatty acid profiles, expression of fatty acid desaturase 2 and selected immune system-related genes in the offspring of gilthead seabream. Br J Nutr 122:25–38. doi: 10.1017/S0007114519000977

Turkmen S, Perera E, Zamorano MJ, et al (2019b) Effects of dietary lipid composition and fatty acid desaturase 2 expression in broodstock gilthead sea bream on lipid metabolism-related genes and methylation of the fads2 gene promoter in their offspring. Int J Mol Sci 20:6250. doi: 10.3390/ijms20246250

Turkmen S, Zamorano MJ, Fernández-Palacios H, et al (2017) Parental nutritional programming and a reminder during juvenile stage affect growth, lipid metabolism and utilisation in later developmental stages of a marine teleost, the gilthead sea bream (*Sparus aurata*). Br J Nutr 118:500–512. doi: 10.1017/S0007114517002434

Vagner M, Robin JH, Zambonino-Infante JL, et al (2009) Ontogenic effects of early feeding of sea bass (*Dicentrarchus labrax*) larvae with a range of dietary n-3 highly unsaturated fatty acid levels on the functioning of polyunsaturated fatty

acid desaturation pathways. Br J Nutr 101:1452–1462. doi: 10.1017/S0007114508088053

Vagner M, Zambonino Infante JL, Robin JH, Person-Le Ruyet J (2007) Is it possible to influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a nutritional conditioning during larval stage? Aquaculture 267:165–174. doi: 10.1016/j.aquaculture.2007.01.031

Vassallo-Agius R, Watanabe T, Yoshizaki G, et al (2001a) Quality of eggs and spermatozoa of rainbow trout fed an n-3 essential fatty acid-deficient diet and its effects on the lipid and fatty acid components of eggs, semen and livers. Fish Sci 67:818–827. doi: 10.1046/j.1444-2906.2001.00328.x

Vassallo-Agius R, Watanabe T, Yoshizaki G, et al (2001b) Quality of eggs and spermatozoa of rainbow trout fed an n-3 essential fatty acid deficient diet and its effects on the lipid and fatty acid components of eggs, semen and livers. Fish Sci 67:818–827. doi: 10.1046/j.1444-2906.2001.00328.x

Vetter R, Hodson R, Arnold C (1983) Energy-metabolism in a rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*). Can J Fish Aquat Sci 40:627–634

Vickers MH, Breier BH, Cutfield WS, et al (2000) Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol-Endocrinol Metab 279:E83–E87. doi: 10.1152/ajpendo.2000.279.1.E83

Vickers MH, Breier BH, McCarthy D, Gluckman PD (2003) Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. Am J Physiol-Regul Integr Comp Physiol 285:R271–R273. doi: 10.1152/ajpregu.00051.2003

Volkman JK, Jeffrey SW, Nichols PD, et al (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219–240. doi: 10.1016/0022-0981(89)90029-4

Wang X, Devaiah SP, Zhang W, Welti R (2006) Signaling functions of phosphatidic acid. Prog Lipid Res 45:250–278. doi: 10.1016/j.plipres.2006.01.005

Watanabe T, Ohhashi S, Itoh A, et al (1984a) Effect of nutritional composition of diets on chemical components of red sea bream broodstock and eggs produced. Nippon Suisan Gakkaishi 50:503–515. doi: 10.2331/suisan.50.503

Watanabe T, Takeuchi T, Saito M, Nishimura K (1984b) Effect of low protein-high calory or essential fatty acid deficiency diet on reproduction of rainbow trout. Nippon Suisan Gakkaishi 50:1207–1215. doi: 10.2331/suisan.50.1207

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Rev Fish Biol Fish 6:259–286. doi: 10.1007/BF00122583

Wiegand MD, Johnston TA, Martin J, Leggett WC (2004) Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Can J Fish Aquat Sci 61:110–121. doi: 10.1139/f03-146

Wilke CO (2020) cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2." Comprehensive R Archive Network (CRAN)

Yang Y, Pham TX, Wegner CJ, et al (2014) Astaxanthin lowers plasma TAG concentrations and increases hepatic antioxidant gene expression in diet-induced obesity mice. Br J Nutr 112:1797–1804. doi: 10.1017/S0007114514002554

Zakeri M, Marammazi JG, Haghi M (2014) Dietary indispensable amino acid concentrations based on amino acid profiles of broodstock, eggs and larvae in yellow fin seabream, *Acanthopagrus latus*. J Persian Gulf 5:1–14

Zambonino-Infante JL, Panserat S, Servili A, et al (2019) Nutritional programming by dietary carbohydrates in European sea bass larvae: Not always what expected at juvenile stage. Aquaculture 501:441–447. doi: 10.1016/j.aquaculture.2018.11.056

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